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Systems for sale; way of life comes free

FOR as long as nations have been going to war with each other, capturing the market of another country has been regarded as a desirable alternative to the bloody business of capturing its virgins, chests of gold, galleons or surface-to-air missiles. And whilst a favourable trade balance made up from a portfolio of exports ranging from pins to double-decker buses is a fairly good way of making a possible enemy into an ally out of necessity, a much better way, most governments seem to agree, is by exporting whole systems as opposed to single items.

The virtue of your system in someone else's territory is pretty obvious. First it needs installing; not really a job that the locals can do, so hordes of technicians are temporarily exported to roam unchallenged in the course of their technical duties. Then the locals have to be taught how to keep it going; schools are established and, with luck, some of your own way of life and thinking rubs off, along with all the know-how. Then there will be a continuing demand for spares –available, of course, only from the original manufacturers if the design is made exotic enough. There will be a steady stream of advisers, consultants, conferences and working parties. And when the system wears out, guess who has just the right second-generation system, tailor-made to requirements and ready to start the cycle all over again. It is clearly much more than just foreign exchange which such an arrangement brings in –it provides cultural links, a terrestrial toehold and, of course, political benefits.

In the past these super-exports have included legal systems, constitutions, drinking habits, land drainage, agricultural practices, railways, literacy schemes, communications networks, family planning programmes, meteorological services, health care, airlines and, inevitably, military systems of all shapes and sizes. Nor is there any sign that the pace in systems-exporting will slacken; indeed the newly-rich oil countries are a ready-made market, as every industrialised country knows. But what, besides the staple military diet, is likely to be

an attractive system to sell in the 1980s and 1990s? One completely new item is likely to be the earthquake early-warning system.

Few can now be ignorant of the remarkable strides taken in earthquake prediction in the past ten years. Very few quakes have yet been accurately predicted, but the pace of scientific advance and the lessons now being learnt about public response in various parts of the world to the issuance of warnings make it almost inevitable that over the next few years a few countries, notably China, the Soviet Union, Japan and the United States are going to make major strides in learning how to save lives. But many of the most predictable earthquakes probably occur in other parts of the world—Turkey, Iran, the Philippines, Mexico, Central America, Peru, Chile and so on, and many of these countries look very attractive propositions to the politically-oriented travelling salesman.

A national earthquake warning system will require hundreds of widespread installations, the development of a data network and a lot of involvement of the local inhabitants, especially if the more unusual predictions of earthquakes, such as animal behaviour, assume importance. It will be seen to be a worthy altruistic enterprise, proof that the government is concerned. It will also cost a lot of money. It looks absolutely made-to-measure as an exportable system. The question is not so much whether but how.

If the 's' in its name means anything at all, there is surely a strong case for Unesco to take a long look at the problem. For although warning systems would undoubtedly be good things for many countries, these same countries can do without latter-day economic imperialism, often in the guise of overseas aid. An alternative means of delivering effective systems without the imperialist content will not be easy to find, but it is at least worth making a serious effort to do so at this stage. For time is getting short. □



Overdue: another scientific revolution

J. Tuzo Wilson, Director General of the Ontario Science Centre in Toronto, argues the case for a return from growth to stability

CERTAIN major turning points in the history of science can be called scientific revolutions. Their influence is not confined to science, for they have solved philosophical puzzles and changed economies, but their importance warrants a search for new revolutions. It might be fruitful: history shows that earlier scientific revolutions have cast their shadows before them and have progressed through several parallel stages.

Before each of the past revolutions, for example, their old theories worked well and were useful. Their value has been partly hidden by subsequent attempts to denigrate them: before Copernicus, Ptolemaic astronomers had invented the calendar, predicted eclipses and acted as navigators for Columbus and Magellan; biology, classical physics and geology were similarly useful and well-developed sciences, even before the revolutionary discoveries of Darwin, Becquerel and Wegener. The triumph of each of the new theories was due to important new discoveries, observations or ideas which the old theories could not explain, as the observation of the phases of Venus, the discovery of radioactivity and realisation of the significance of mid-ocean ridges and paleomagnetism all demonstrate.

In each case the great event marking the revolution revealed contradictions and introduced problems which the old theory could not resolve, leading to a period of confusion. For the most part it was not experts but rebels, outsiders and interlopers from other fields of science who suggested the need for a revolution and produced the evidence supporting it. Thus most geologists continued to oppose the concept of continental drift long after Wegener (a meteorologist) had championed it, after physicists had provided key evidence supporting it and after many of the public had accepted it.

So, far from welcoming the new ideas, the establishment, who had the most to lose, clung for as long as possible to the old, justifying their position by questioning the new data, discrediting those who advanced them, and trying to patch up the old theories. In these endeavours they frequently found themselves supporting quite illogical positions. Before each revolution was accepted, the state of the subject had become chaotic, but each solved many problems so that the ensuing periods were times of great scientific progress

and material benefit.

When one now looks for a field ripe for a scientific revolution the only likely candidate appears to be the marginal science of economic theory. It seems to meet most of the criteria advanced. To the extent that the steady increase in wealth over the past few centuries can be attributed to theory, economic theory can be held to have been successful. But any examination of nature or of primitive societies or any consideration of the mathematical consequences of continuous growth will show that stability is normal and growth abnormal. The history of the West since the Renaissance, and particularly since the start of the Industrial Revolution, has been one of growth. It has made most of us forget that this is unusual and must end.

Now an event has occurred which is so important that it is altering the course which history has maintained for several centuries. The cause of the period of growth and the reason for a delay in fulfilling Malthus' predictions has been that for several centuries energy has become ever more abundant at ever less cost. The oil embargo and the quadrupling of the price of crude oil has ended this period and marks a turning point in history. Some say we must seek substitutes. This is not as important as many seem to think, because many are already available. What is needed is the recognition that no substitutes, either known or awaiting discovery, are at all likely to be so cheap. It seems probable that holes drilled in the ground to produce petroleum have been cheaper than any mines, factories or collecting devices which can be built to generate other forms of power are likely to be.

But matters go further. The old system has broken down. There is a recession from which economies are not emerging as the old cyclical pattern predicted. There is unemployment coupled with inflation. In addition, many organisations and individual members of the general public since Malthus have recognised the coming breakdown, seen that growth can only be temporary, and predicted change. It is not right to say that history has proved Malthus wrong. Fundamentally his ideas were correct, but living in the eighteenth century he did not see that development of power from steam, electricity and internal combustion engines and the whole industrial revo-

lution would delay their impact.

Moreover, the establishment, blind to logic, denigrates those who foresee the inevitability of change as "doomsters", and clings to theories which if they have not broken down completely already are mathematically certain to do so before long. The differences between those who advocate growth and those who predict no-growth is not as great as might be imagined. The supposed economic experts all demand a return as quickly as possible to an annual rate of growth of, say, 5%. Politicians, businessmen and labour leaders all say that this can be attained, and they well may be right for the next year or two, which is as far as they look ahead, to the next election, next meeting of shareholders, or next signing of a union contract.

On the other hand, any accountant can calculate that an increase in demand of 5% annually will in thirty years produce more than a fourfold increase in demand. Thirty years is a period most people can expect to see, and one for which young people make plans, but a fourfold increase in demand is ridiculous in face of present shortages. Thus the position held by the present ruling establishment, as has happened in past revolutions, has become untenable. As an example, I was astonished that an influential man to whom I advanced these views replied that they could not be right because if true they would ruin business!

It will be difficult to convince businessmen, who for several centuries have flourished upon growth, that growth cannot long continue. It will be particularly distressing to them because in the past the declining cost of energy has rewarded innovation with profit, so that greed overcame man's natural lassitude and aversion to change. Each change—from slaves to animal power, from windpower to steam and from steam to internal combustion engines—increased profits.

In future the changes to more expensive forms of power will decrease profits and this is producing a tendency to cling to existing practices. If supplies become exhausted before this is overcome and adequate replacements made, breakdown and anarchy are likely to result. Therefore it is vital to prepare for a return to a stable society and not fritter away resources which are still available to us trying to stimulate growth when the means to support growth are no longer going to be available.

For each past revolution, however, recognition of the new theory has

always made progress possible again. Even after it has been recognised that we must accept an overall economy which is less lavish than before, great rebuilding will be needed. As the supplies of oil and gas run out the whole petroleum industry, for example, will have to be replaced by other industries, ultimately by ones that depend upon renewable resources. We have seen many such changes, for example the change over the past 50 years from coal to oil. These changes involve work and employment.

No growth overall need not mean overall stagnation once the correct direction is recognised and followed. Undisturbed Nature is stable, but it is

certainly active. I therefore prefer the term renewal economy to no-growth society. The best chance to avoid ultimate breakdown and anarchy and to maintain any of the present forms of society is to take steps at once to change them. It should be noted that the change in philosophy must be farther reaching than a mere change between one or another existing political party or between a planned society and free enterprise. All the present economic philosophies ignore Nature's and mathematics' edict that growth cannot continue indefinitely. If some extreme governments have achieved conditions of no growth in their countries it is because of their

ineptitude, not their desire.

The need is imperative to recognise and to start to implement this great revolutionary return from growth to stability. Its effects are likely to be more traumatic than those of any previous scientific revolution, but can best be ameliorated by early recognition and acceptance. Nearly three years have already passed since the revolutionary turning point in the availability of energy occurred. It is ironic that it would appear that it is the conservative blindness of those who should be leaders, whether in politics, business, or labour, that is the greatest impediment to advance.

Space: a new phase?

Keith Runcorn and Paul Coleman describe plans for a major new effort in exploration and urge more European cooperation

THE landings of Viking spacecraft on Mars on July 20 and September 4, 1976, and of Venera 9 and 10 on the surface of Venus in 1975, mark the culmination of a period of lunar and planetary exploration. Over the past decade manned Apollo landings and the unmanned Ranger, Surveyor, and Luna 16, 20 and 24 landings have been made on selected sites on the Moon. From the data collected on all these remarkable missions, our understanding of the evolution and present state of the Moon and planets is developing at an unprecedented rate. The next decade will see a revolution in our knowledge of the solar system comparable with those studies of continental drift and plate tectonics which, over the past two decades, have transformed our understanding of the Earth.

Europe, with a combined gross national product greater than that of the USA and the Soviet Union, has not so far taken a role which reflects its strength in this field, one of mankind's greatest explorations and scientific endeavours. An opportunity will be afforded by the International Solar System Programme, a project of international collaboration in the exploration of the solar system. The International Council of Scientific Unions recommended last year that all countries should consider how they can most effectively contribute.

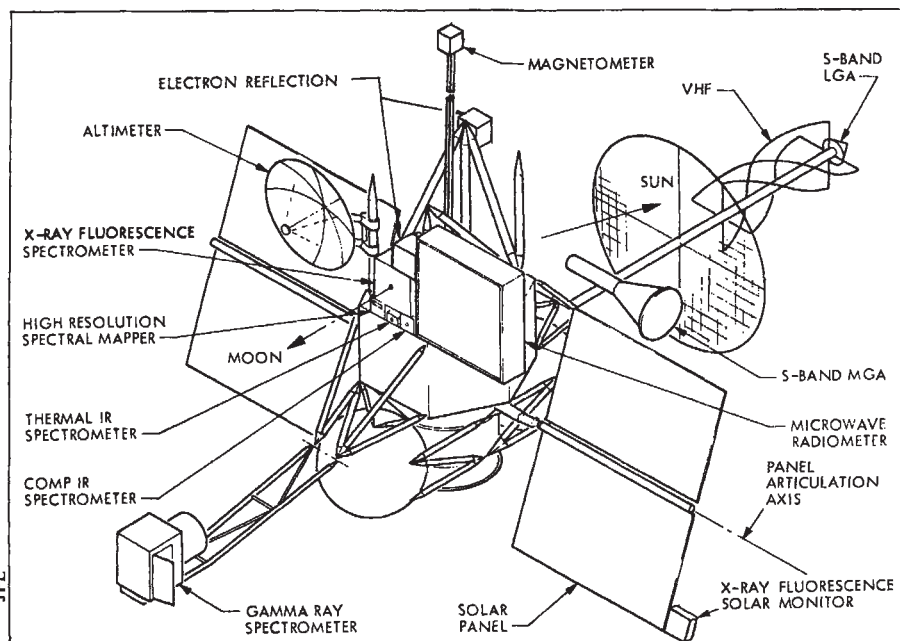
Our knowledge of the Moon exceeds

in its detail that of any other body in the solar system except the Earth. While it might be feared that sampling the Moon in a restricted number of sites might hopelessly bias our interpretations, it is now clear that the absence of an atmosphere and an ocean and the weaker tectonic forces than on Earth have resulted in much less segregation and concentration of elements and a much smaller variety of rock strata than on the Earth. This has in turn made it possible for the relatively small number of sampling sites to yield data reasonably representative of the Moon as a whole. On terrestrial planets

limited samples might not be as successful in determining global properties.

The Moon differentiated about 4.4×10^9 years ago, forming an anorthositic gabbroic crust apparently thicker on the far side than the near side. The sources of the mare basalts, (crystallisation ages 3.2 to 3.8×10^9 years) which filled the great impact basins left in the Moon after catastrophic bombardment, seem to have differentiated at the same time. The processes that brought about this differentiation are obscure and the depth to which it extended is unknown. If the Moon is found to possess an iron core, this early differentiation must have occurred throughout the Moon. The siderophile and volatile elements on the Moon are depleted relative to the Earth and meteorites: information potentially a key to our understanding of the early evolution and origin of the Moon.

Three entirely unexpected charac-



Flight configuration for TBOL

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teristics were established from physical studies: large positive gravity anomalies over the circular mare (the mascons), moonquakes both deep and shallow, and widespread magnetisation of the crust. The interpretations of these discoveries are still controversial but we conclude that in its early history the Moon was much more active internally than it is today. If so, why has the Moon died?

The interpretation of gravity field on the near side requires an equally complete survey on the far side. Basins of comparable size to the mare are known to exist on the far side, but they are not filled with lava and whether they have negative or positive gravity anomalies is uncertain. Thus, the exact

delineation of gravity above these far-side basins is of critical importance to the understanding of the process which resulted in the flooding of mare and the production of mascons on the near side.

Again, the controversy concerning the interpretation of the magnetism of lunar rock centres upon whether the moon generated, between 4×10^9 years and 3.2×10^9 years ago, a general magnetic field which it does not have today. The direction of magnetisation of the lunar crust was impossible to determine by the methods used in terrestrial paleomagnetism; the Apollo samples were unoriented. Instead, interpretation of magnetic anomalies must be used. The existence of an iron

core in which a dynamo process might once have been active remains in doubt. A more accurate value of the moment of inertia factor (0.4 for a uniform sphere) which is now given as $.392 \pm .003$ could resolve this particular matter.

The definitive solutions of many interesting problems raised by existing data depend on completing the survey of the moon which has been so well begun. To this end the lunar polar orbiter has been conceived in a mission named at present the Terrestrial Bodies Orbiter-Lunar (TBOL). A satellite carrying a variety of remote sensors would be put in lunar orbit about 100 kilometres above the surface so that a complete and relatively detailed sur-

The experimental programme

The geochemical experiments consist of a solid state (germanium) counter for gamma rays and four proportional counters (three looking at the moon and one at the sun) for X rays. The former will detect both the radioactive nuclides K, Th, U and the elements Fe, O, Si, Ti, Mg, Al, Ca (in declining order of detectability) excited by the galactic cosmic-ray neutrons and by solar flares. The latter will detect the characteristic X rays emitted from Al, Si, Mg for the quiescent sun and also Ca, K, Fe, Ti during flares. The sampling depth will be 15 cm for the gamma rays and 10 μ m for the X rays and spatial resolutions will be 50 km and 10 km, respectively. These experiments will provide much more detailed elemental abundance data than has so far been available and will contribute to the understanding of the differentiation of the moon, especially of the highland regions thought to be anorthositic gabbroic in character but only on the basis of fragmentary samples.

Water of the moon, perhaps retained in the regolith of deep craters near the poles, is exciting much interest and the gamma ray experiment may detect it from the 2.22 MeV line de-excitation of deuterium produced by slow neutrons created in material on the lunar surface by cosmic-ray bombardment.

The radioactivity is known to be greater in the western mare, so that an experiment to detect the surface heat flow is proposed. It may be surprising that this can be done from orbit, but microwave radiometers (3 cm, 10 cm, 20 cm)—three identical beams with 50 km resolution—detect the black body radiation from different depths (down to about 5 m) in the regolith where, because of its ex-

tremely low conductivity, the vertical temperature gradient is large. These radiometers and infrared detectors will also measure the lunar daily inflow and outflow of solar radiation, from which the thermal conductivity of the regolith can be calculated. Thus the distribution of heat sources within the moon will be globally mapped and hypotheses such as convection in the interior will be tested.

Reflectance spectroscopy techniques over wavelengths 0.35–2.5 μ m will be used to determine the mineralogical composition. This method has already been used effectively to determine the composition of the major satellites of planets. The wavelength of absorption lines from the excitation of electrons from Fe^{++} , Fe^{+++} and ions of other transition metals are influenced by the crystalline structure of the SiO lattice. It will be supplemented by spectro-stereo images with six filters. The latter instrument, of course, will also provide, in high resolution (~ 100 m), further details of the morphology, stratigraphy and, through crater counts, chronology of the lunar surface.

Magnetic maps of the moon will be obtained at the orbital altitude by a 3-component magnetometer accurate to 0.1γ and at the surface by an electrostatic analyser of electron fluxes. The latter will detect low energy electrons, spiralling around the lines of the solar wind magnetic field, which are reflected from the convergence of the field lines produced by surface magnetic anomalies. These two methods will complement each other as crustal magnetic anomalies having sharp contrasts of magnetisation look very different at the surface and at heights of 100 km. As various interesting explanations of

the magnetisation of lunar rocks have been suggested, some involving cometary and meteoritic impacts, data on the general morphology of magnetic anomalies may establish whether or not these external sources or a magnetic field originating at the lunar core are responsible.

The existence of a lunar core can also be investigated by observing the electromagnetic response of the moon as it enters the fairly steady geomagnetic tail field. The core acts initially as diamagnetic and then the induced dipole moment begins to decay—from this initial decay the free decay time constant of the electric currents in the core can be determined.

The map of the gravity field on the near side will be improved by tracking of the orbiting satellite. For the far side of the moon, complete maps will be made of the gravity field, of which at present nothing is known except by inference from the evolution of the orbital elements of the subsatellites launched in the Apollo 15 and 16 missions. The closely related study of the figure of the moon has been controversial, as the coverage of the laser altimeters on the Apollo 15, 16 and 17 missions has been very limited and the classical earth-bound method, using the geometrical librations which enable height determinations to be made by the parallax principle, were extremely noisy. The radar altimeter in the orbiter should enable the surface to be mapped completely to a high accuracy. The moon departs from a sphere in a variety of ways and the mapping of the moon will be very important to refine our understanding of its non-hydrostatic shape.

vey would be possible. A radio-relay satellite would be put into a more distant orbit to provide an almost continuous radio contact between the instrumented polar orbiter and the Earth. Such a mission, in which the scientific aspects have already been developed (see figure and box), is under consideration by NASA for a 1982 launch (see following story).

The Terrestrial Bodies Orbiter-Lunar mission is part of a new phase of

planetary exploration, by means of remote-sensing orbiting spacecraft. A somewhat similar approach, but with a spinning spacecraft, is now under way in the United States for exploration of Venus, and a Jupiter orbiter is in the planning stages. These latter two missions will also involve atmospheric probes, which are unnecessary for planets and bodies without a substantial atmosphere. Modified versions of the TBOL spacecraft could extend

further the exploration of such bodies in future.

More active European participation at this crucial stage in the exploration of the solar system would be timely and would enable European scientists, who contributed so much to the analysis of lunar samples, to revive the old scientific tradition of which we have been reminded in recent years by the anniversaries of Copernicus, Kepler and Galileo. □

USA

Ford's parting gifts

Colin Norman in Washington reports on the proposals for research and development in Mr Ford's third and final budget

WITH just three days of his brief and troubled Presidency left to run, Mr Ford this week presented Congress with a complex and detailed set of budget proposals for the 1978 Fiscal Year, which begins on October 1, 1977, more than eight months after he leaves office. Though they are likely to be substantially revised, both by the incoming Carter Administration and by the Democratic-controlled Congress, Mr Ford's parting economic proposals could result in substantial increases in support for science and technology, particularly for basic research.

The proposals represent the lame-duck Administration's detailed prescriptions for limiting growth in Federal spending, reforming the sprawling government bureaucracy and setting priorities among Federal programmes. During the next few weeks, President Carter and his economic advisers will sift through Mr Ford's budget and offer a raft of amendments to bring it into line with the new administration's economic and political philosophies. Carter will concentrate on the more political proposals, however, and it is likely that many of the less controversial programmes, which include most concerned with science and technology, will survive the change of administration more or less intact.

Mr Ford's budget includes a total of about \$26,300 million for research and development, an increase of about 8% over estimated expenditures this year. As in the past few years, defence and energy research and development account for the bulk of the increases, but basic research is also singled out for favourable treatment, rising by about 9%, to reach some \$3,000 million. Inflation is expected to hover around 6%, so the Ford budget proposals should result in a modest, but real,

growth in Federal research support.

According to a discussion paper published by the Office of Management and Budget, funding for basic research in the United States has declined by about 27% over the past decade, chiefly because funding increases have not kept pace with inflation. Although the document suggests that basic science in the United States is competitive with that of other countries, the decline in support "has raised concerns about whether the United States might not be underinvesting in basic research to the detriment of the nation's future".

The chief beneficiary of the proposed increases is the National Science Foundation (NSF), scheduled for an increase in funds for some 12%. According to Richard Atkinson, the acting director of NSF, a major use of the proposed increase will be to upgrade scientific instruments and equipment in universities and colleges. NSF's total budget is set to climb to \$885 million under Mr Ford's proposals.

The budget is the first to be produced since the establishment of the White House Office of Science and Technology Policy (OSTP), and some credit for the proposed increases is being claimed for the new arrangement. H. Guyford Stever, the head of OSTP and Mr Ford's science adviser (who will also leave the Federal Government this week) notes with some satisfaction that "although it is a constrained budget, it is not as constrained as in previous years". Asked whether he expects the Carter Administration to go along with the proposals, Stever pointed out that few of the research and development proposals have political implications, and he suggested that "if the incoming administration asks for advice on the research and development budget it would get much the same advice" as Mr Ford received. Since Mr Carter had not chosen a replacement for Dr Stever at the beginning of this week, he does not seem to feel an urgent need for such advice.

Included in the budget proposals are a number of new programmes which have been waiting in the wings for some time, and which are likely to meet with approval from the Carter Administration. Prominent among them is a major effort in earthquake research. A three-year project divided between the National Science Foundation and the Department of the Interior, it is expected to receive about \$150 million, with the first instalment of \$23 million coming this year. The goals are "development of a reliable prediction capability within 10 years through increased research on fundamental causes of earthquakes and precursor phenomena", establishment of building codes for structures in earthquake prone areas, publication of regional hazard assessment maps, and studies of the social, legal and economic implications of reliable earthquake prediction capability. Such a programme was recommended last year by a White House Advisory Committee, and a similar effort was incorporated in a bill passed last year by the Senate. The Carter Administration is unlikely to reject the proposal.

Another area to receive some long-overdue support in Mr Ford's budget is agricultural research. Some \$28 million has been proposed for a new programme of basic research on photosynthesis, nitrogen fixation, crop productivity, and genetic engineering of crop plants, funded through a system of competitive grants similar to those operated by the National Science Foundation and the National Institutes of Health. At present, virtually all the research supported by the Department of Agriculture is funded through block grants to institutions, and initiation of a competing grants programme has long been recommended, particularly by committees of the National Academy of Sciences, as a way to increase the quality of agricultural research. The proposed programme is virtually certain to receive a warm reception in Congress since the House last year approved a bill designed to initiate just such a programme.

Table 5 Conduct of basic research by major departments and agencies (in millions of dollars)

Department or agency	Obligations			Outlays		
	1976 actual	1977 estimate	1978 estimate	1976 actual	1977 estimate	1978 estimate
Health, Education and Welfare (National Institutes of Health)	660 (590)	744 (659)	796 (710)	694 (627)	644 (568)	745 (662)
National Science Foundation	541	612	688	534	574	650
Energy Research and Development Administration	346	389	427	330	370	413
National Aeronautics and Space Administration	298	352	365	297	321	360
Defence—Military functions	248	274	314	225	279	314
Agriculture	171	193	215	168	200	210
Interior	121	127	143	125	125	137
Smithsonian	26	31	32	28	31	31
Commerce	22	25	24	21	24	25
Environmental Protection Agency	14	21	18	13	21	14
All other	16	17	19	16	15	20
Total	2,463	2,785	3,041	2,451	2,604	2,919

Space science is also singled out for a modest increase in Mr Ford's proposed budget. A new start has been proposed on the construction of the Large Space Telescope (LST), a 2.4-metre optical telescope which is expected to be launched by the space shuttle in 1983. Estimated to cost a total of between 435 and 470 million dollars, the LST has consistently been ranked as a top priority space science project by the National Academy of Sciences, but restrictions on federal expenditures in the past two years have twice caused the programme to be deferred. Funds have also been proposed for another high-priority mission, construction of a spacecraft to orbit Jupiter and send probes into the planet's atmosphere. And finally, Mr Ford's budget includes funds to begin construction of three more shuttle orbiters, in addition to the two which have already been approved. There are, however, some casualties in NASA's budget, most prominent of which is a proposal to place a spacecraft in a polar orbit around the moon (see page 197). A start on that mission was anticipated in the 1978 Fiscal Year, but it has been deferred, at least until 1979. So far, Mr Carter has made few public comments about the space programme; thus the fate of Mr Ford's proposals for NASA are uncertain. The projected total costs of the LST may, however, make it a plum target and there is some speculation that Carter will ask NASA to seek foreign financial participation in the project.

One area of Mr Ford's research and development budget which is likely to be substantially modified by the Carter Administration is the proposed funding for energy research and development. Though Mr Ford has recommended a hefty increase in total support for energy programmes, with some \$3,000 million included in the budget for research and development, much of the additional money would be swallowed up by nuclear energy research efforts. The Liquid Metal Fast Breeder Reactor

(LMFBR) programme alone is scheduled to increase from 686 to 855 million dollars. During the election campaign, Carter promised to reduce the priority of the LMFBR project and to seek international participation in it. He also consistently promised to try to reduce United States' reliance on nuclear power.

A particular focus of conflict between Ford's energy proposals and Carter's election promises is likely to involve programmes designed to increase energy conservation and to spur commercial introduction of solar heating devices. Mr Ford's budget would involve virtually no increase in funds for research and development on energy conservation, chiefly because the Ford Administration believes such efforts should be the responsibility of private industry rather than the federal government. Similarly, the proposed Ford budget would reduce federal support for a programme designed to provide subsidies for the production and installation of solar heating devices. Again, the argument is that the technology is relatively well developed and private industry should bear the chief responsibility for its commercialisation. Throughout the election campaign, Mr Carter frequently promised to make energy conservation and the development of alternative sources of energy his top priorities. Moreover, James Schlesinger, the former head of the Atomic Energy Commission, whom Carter has named as his chief energy adviser, has also argued strongly for increased efforts to stimulate energy conservation. The thorny question of government involvement in private industry, and Federal subsidies to spur commercial development of those technologies, will clearly have to be addressed very soon by the new president and his energy team. As for fusion energy, Mr Ford's budget provides funds for construction of a large Tokamak device at Princeton University—a project which was reported to be in jeopardy at one stage in the budget

deliberations—and it also contains funds to increase the already sizeable fusion project at Lawrence Livermore Laboratory.

Another area which is likely to be a source of controversy is support for biomedical research. The Ford budget provides very little increase in support for biomedical research funded by the National Institutes of Health (NIH), and in particular it seeks to hold the budgets of the National Cancer Institute and the National Heart and Lung Institute approximately constant, after several years of rapid growth. There is also a proposal to transfer responsibility for NIH's intramural research to the Assistant Secretary for Health, a suggestion which is likely to raise considerable protest from biomedical researchers since it smacks of increased political control over health research.

Ford tried to hold down spending by NIH in his two previous budgets but Congress has refused to go along with the reductions and instead it has voted hefty increases for biomedical research. The same pattern is likely to be repeated this year, but the Carter Administration will soon be forced to take a close look at priorities in biomedical research. In that regard, a study produced last year by a presidential commission recommended that there should be some changes in the privileged political position of the National Cancer Institute, but the recommendations have so far been neglected.

Finally, it is worth noting that when President Ford entered the White House in August 1974, he inherited, among other troubles, a scientific community which had been complaining bitterly about declining research support and the lack of a central science policy office. In his brief term of office, Ford has taken several steps to attend to the concerns of the scientific community, and when he leaves the White House this week, he will bequeath his successor a much more healthy scientific legacy. □

SWEDEN

Reforming the Councils

Wendy Barnaby reports from Stockholm on proposals to reform Sweden's Research Councils

THE reform of Sweden's Research Councils due to go into effect on July 1 this year is basically an attempt to set up a structure which will accommodate both society's need for research that produces usable and useful results, and the scientists' traditional demand for freedom in conducting research. Of course, any attempt to fuse these two is only as good as the mechanisms whereby fusion is to be brought about—and the will of the people involved to make it stick. But the mechanisms, at least as explained

by Mr Jan Stiernstedt, Permanent Secretary of the Ministry of Education and chairman of the reform's organisational committee, look like a model example of the Swedish genius for balance and compromise.

Within the sphere of the Ministry of Education there are at the moment five Research Councils (Humanistic, Social Science, Medical, Atomic and Natural Science), with an average membership of eighteen plus a chairman. In the budget year 1975–76 they were allocated about 6% of the state's expenditure on research and development, which itself made up 3.3% of the total budget, so they are hardly financial giants. In fact, as far as research and development goes, they embody precisely that weighty meagreness which is the essence of prestige

—and that they certainly have. Their various structures have resulted from haphazard growth reflecting the enormous post-war increase in the breadth and complexity of research; the reform aims to tidy them up organisationally as well as to make them more sensitive to public interests.

It envisages only three councils (Humanistic and Social Science, Medical and Natural Science), each with ten members who will hold office for six years, and a chairman. The actual number of members will thus have been reduced to a third of its present total, an attempt to make the new bodies more effective. At the same time, however, they are to be made as representative as possible of the views of the academics in each field. Seven members of each council are to be elected from the universities and institutes of higher education, where small assemblies will be elected by those members of staff who have a

USSR

● The recent announcement that the Soviet Union will sell 200 tonnes of heavy water to India for use in the Rajasthan Atomic Power Plant reveals a certain modification in Soviet policy, regarding both aid to India and the non-proliferation of potential nuclear armaments.

Although the first Soviet-Indian mutual cooperation agreement is now more than five years old, India has until now received relatively little technological aid from the Soviet Union, either as hardware or expertise. During the visit of Mrs Indira Gandhi to Moscow in June, 1976, the 'strengthening' of such cooperation was one of the main topics of discussion. The heavy water sale would appear to be one of the fruits of these talks.

Heavy water is, of course, a sensitive topic as far as India is concerned. Although she can produce a small amount of her own, this is not sufficient to supply an installation the size of the Rajasthan power station. Originally, India obtained heavy water from the USA and Canada; the connection with India's explosion of a nuclear device led Canada to cut off supplies, and later to make the ban permanent following unsuccessful negotiations on inspection and safeguards.

The Soviet Union has always shown considerable caution regarding nuclear exports. A recent *Pravda* article, while not referring directly to the Indian deal, emphasises the role of

the Soviet Union, within the IAEA, the "London Club" of nuclear suppliers and the United Nations, in "organising monitoring operations". The article stresses the need to increase the efficiency of such international control, and quotes the Soviet non-proliferation memorandum to the 31st UN General Assembly emphasising the "special responsibility" of the nuclear powers and the need for "strict guarantees that international cooperation in the peaceful use of nuclear energy should not become a channel for the proliferation of nuclear armaments".

The Soviet Union has presumably found some way to extract from India the kind of guarantees which, after two years' negotiation, Canada was unable to obtain.

● The filling of the Nurck reservoir on the river Vakhsh in Tadzhikistan has been halted at the 140 m mark on the 300 m dam, on the advice of a joint Soviet-US geophysical team now working in Tadzhikistan under the terms of the Nixon-Brezhnev co-operation agreement.

Commenting on the decision, the Academic Secretary of the Soviet Academy of Sciences, I. Ivanov, noted that the dam is situated in the only region of the continental part of the globe where as many as 2,000 tremors occur annually. Filling of the dam has produced a marked increase in seismicity and, according, further raising of the water-level will take

place very gradually. There is no suggestion, however, that the planned final capacity of the reservoir will be reduced.

● One of the anomalies of Dr Veniamin Levich's position as a *refusenik* is that although he is no longer permitted to work as a scientist he still retains his position as a Corresponding Member of the Academy of Sciences. The Academy itself still retains a certain autonomy, and according to its statutes requires a two-thirds majority vote in secret ballot for the expulsion of a member. When an Academy meeting to discuss draft changes in its rules was called for December 22, 1976, Academician Levich wrote to say that he wished to speak at the meeting, to propose a statement clarifying the moral responsibility of Academy members. He was informed in due course that the said meeting had been postponed until February 1977, "when this proposal will be entered".

In proposing the statement, Levich clearly wished to draw attention to the plight of dissident scientists. It should be noted, however, that the last time a number of Soviet Academicians made a declaration on 'moral responsibility'—as individuals, and not as a corporate body—was during the 1973 press campaign against Andrei Sakharov. They declared then that the first duty of a Soviet scientist was uncompromising support for the State.

Vera Rich

PhD. These local bodies will together form a national assembly, which will then elect the council members. The other three members will be appointed by the government and will represent the various official bodies that exist here within each field: the Environment Protection Board and the Board of Education, for example, within the humanities and social sciences. The chairman will be appointed by the government. Each council is thus to embody the principle of scientific freedom by having members drawn only from within the field. The fact that a majority of members will be elected by active researchers will bend the councils in the direction of current interests.

But what happens if enthusiasm for current research interests solidifies into unwillingness to risk failure with newer or simply unfashionable ideas? This is a criticism made by one prominent Swedish medical scientist of the existing Medical Research Council. It is precisely to prevent the conventional

wisdom of the dominating group within any field from restricting other impulses that the reform was undertaken. And as the impulses to be encouraged are those which come from the broader society, the main innovation of the reform is the creation of a special body to give them a persuasive voice—the Research Councils Coordinating Board.

This will exist alongside the councils and have two main tasks: to co-ordinate administrative procedures amongst them and to promote cross-disciplinary research projects of interest to society in general and needing cooperation between the different councils, institutes and boards. It will consist of a chairman, to be appointed by the government, and twelve other members, one to be appointed from each of the three councils, the Agricultural and Forestry Research Council and the Board for Technical Development, and the other seven to be appointed by the government as elected representatives of the community. At

least four of them are to be members of parliament, and the others will come from local government and trade unions. In order to back up its suggestions, the Board will dispose of funds estimated to total at least Sk30 million (nearly US\$57 million) by the budget year 1979–80.

Attempts to involve parliamentarians in Swedish science policy have so far been remarkably dull. But they have never been underwritten with funds, and money, as Mr Stiernstedt points out, is to be the key to the new Board's influence. If the Board can offer to fund a project, organisational inertia will in many cases be overcome. The Board will be able to stimulate but not dictate; every council will retain the right to decide which projects it will participate in. Finally, then, the scientists—if they want to—will be able to discount the Board's suggestions. On the other hand, they could make use of its resources to widen not only society's outlook, but also their own. □

NETHERLANDS

Satellite plans

Casper Schuurung reports from Holland on the latest Dutch commitment to space research and astronomy

At the end of 1976 the Dutch government finally decided to finance a second astronomical satellite, to be launched by NASA with a Thor Delta rocket in the spring of 1981 from the Western Test Range in California. After the success of ANS, the first Astronomical Netherlands Satellite, in the field of soft and hard X-ray and ultraviolet radiation, the second satellite (IRAS) will do observations in the infrared.

The project started in January 1975 with a preliminary definition study. When contacts were sought for the scientific programme with NASA and the European Space Agency (ESA), there was an enthusiastic response, especially from the US community, at the prospect of stimulating this branch of astronomy. Following a NASA "announcement of opportunity" 13 proposals for experiments came in. A US study team consisting of 11 astronomers started discussions on the IRAS mission with their Dutch colleagues.

At the beginning of 1976 agreement was reached on the scientific programme and NASA expected a memorandum of understanding between the US and the Netherlands to be signed before July, 1976. The Dutch government agreed in principle to the project

in July, in spite of a curtailment of expenditure generally, and it took six months to find the necessary money.

The United States and the Netherlands will both have to pay around 110 million guilders (£1=DG 4.15). Out of the discussions with the ESA, moreover, a British interest arose, and the UK Science Research Council took an active part in the US–Dutch negotiations. Britain is contributing 10 million guilders, for the most part consisting of facilities for flight operations in the Appleton Laboratories in Slough.

The design and building of the satellite represents a challenge to Dutch industry, and especially Philips and Fokker-VFW, which hope to continue the industrial innovation which resulted from the ANS project. A British advisory group, General Technology Systems Ltd, provided evaluation of the possible profits of space activities for the Netherlands in the coming years and of the IRAS project in particular. The results of this study are said to be favourable and support the new project.

The great amount of data from IRAS will make higher demands on storage and retrieval than with ANS. Large scale integration techniques will be used in the on-board computer, giving it a maximum of calculating capacity (500 million bits per day). Also of an advanced design is the extreme cooling system for the tele-

scope and the detectors. Infrared observations are only possible if the instruments are cooled close to absolute zero. A special helium-cooled vessel has therefore been developed.

The results of the IRAS mission, the plan of which was set up in June, 1975 by a commission of 15 American, British and Dutch astronomers, will be received in time to be used for the detailed programming of the experiments with Spacelab in the 1980s. Over a six-month period a survey will be made of infrared sources in the Milky Way and infrared radiation from extra galactic systems. In the following six months more detailed measurements on different sources will be carried out. The astronomers hope to trace and map some 10 million infrared sources. The 925 kg satellite will fly in a circular polar trajectory: one earth orbit will take 124 minutes, so that a sun-synchronous trajectory on a height of 900 km is followed.

Not everybody is as enthusiastic as the astronomers, however. Dutch associations of scientific workers protested in 1974 against further Dutch space activities. At that time an agricultural satellite for underdeveloped countries was under study, as well as the IRAS. This project was not then as far advanced as IRAS, and the benefits for these countries from it were considered small because they lacked the necessary infrastructure. The associations were also against IRAS, as "this type of research would hardly contribute to solve the real problems in the world". □

IN BRIEF

Computing costs

For the second time in three weeks the UK Computer Board for Research Councils and Universities has drawn attention to the problem it is facing with rising costs. In its Sixth Annual Report published just before Christmas (Cmnd. 6696, HMSO, 45p), the Board expressed concern at the rapid increase in recurrent expenditure at university computing centres. These had resulted mainly from staff salary rises and increased maintenance costs. The Board added that it was "unlikely" that all recurrent commitments could be met, and that criteria were being sought for the equitable allocation of the funds available. This is expected to be a tortuous process.

Last week the Board published a report outlining the policy framework the Board considers necessary for the development of university computing

facilities over the next ten years (*Computers in Higher Education and Research; The Next Decade*, HMSO, 60p). Concern about costs was again emphasised but the cause became clearer when a figure was put on the rise. From the 1975-76 level of £6.2 million, the trend is for recurrent costs to rise—"at a rate of 32%". And the figure is an annual one, though the report does not make this clear.

The Board estimates that university computing facilities proposed for the next decade could be obtained at a capital cost of £9.4 million a year. It adds that "it is not entirely clear" that the government's procurement policy favouring ICL has been beneficial "either to the universities or to ICL".

Uranium views

If opposition in Australia to the development of nuclear fuel resources

is successful, scientists who want to become part of the development of uranium mining and processing and of nuclear power will have to leave the country, according to Sir Charles Court, the premier of Western Australia. Responding last week to scientists who have urged the national government to ban uranium mining and exports, he described their attitude as "negative and defeatist".

Aspects of the mining and processing of uranium were the subject of a symposium held in London this week. One presentation, from Michael Davis of the European Commission of the EEC, argued that European uranium needs in relation to the world market were of paramount importance to the market's future. "Close collaboration" between utilities and mining companies was highly desirable, and a contribution from the public authorities was necessary to achieve an orderly market.

It has been said that the warp that holds the complex fabric of science together is peer review, and the woof is the noise made by scientists who complain about it. Be that as it may, it is obvious that without peer review, scientific literature would become a Tower of Babel. One has only to look across the street to see what goes on in the newspapers for reassurance as to this point. Science is essentially hierarchical; its progress and its integrity depend on the existence of an 'establishment', and on the rejection of uncontrolled or unrepeatable experimental results. There are objectors who say that such rigidity prevents valuable innovations from coming to light. The answer to this may be formulated as a Darwinian analogy: such innovations are like the exceedingly rare class of mutations that are beneficial to a species, and hence overcome all odds against their survival and spread.

I am indebted to a lecture by Professor Emilio Segre for an anecdote of Rutherford's rejection, on behalf of *Philosophical Magazine*, of a manuscript by the youthful Bohr, describing a new theory of atomic structure. But the young genius was not to be denied. He journeyed from Denmark to England to confront Rutherford, the giant of physics, and won his point: the famous article was published. The answer to the line in Gray's *Elegy*, mourning the possible loss of "some mute inglorious Milton", may be that Miltons are never mute. Nevertheless, I do not recommend making a trip to the offices of *Nature* to argue with the Editor except under most extreme circumstances.

The word "peer" implies acceptance by a group of equals. The process of acceptance in the group, and acknowledgement of its hegemony by the individual who has been accepted, is complex and fascinating. There are special cases; for example, one of the principles of scientific organisation is

Peer review



THOMAS H. JUKES

that a veterinarian should report only to another veterinarian. I presume that at the head of such a pyramid must be one, perhaps a Dr Cabot, D.V.M., who reports only to the Great Veterinarian. Nobel Laureates are another special case; some of them acquire omniscience after their return from Stockholm, and henceforth they blossom in new fields. This subject is admittedly a delicate one, but I am intrigued by an advertisement in a financial newspaper, appealing for tax-

deductible contributions. "Each contributor will be sent an article on sugar—its effect on the body; an article on Vitamin C—how much to take and how it may benefit your life; and a just-published article showing the dramatic effects of Vitamin C on cancer." Idly, I wondered who reviewed these articles that are so temptingly offered in exchange for cash.

Publishing a book is a way of avoiding peer review. Confronted with criticism, the authors of such books are apt to remind their detractors that Galileo was persecuted by the Inquisition, and that sceptics told the Wright brothers that their aeroplane wouldn't fly. In one recent case, the author said that high-quality DNA and RNA can be supplied from outside the body to "nourish our cells and return them to a healthy state." Apparently he had not heard about nucleases, purine catabolism or gout. The publishers of the book, more interested in sales than veracity, allegedly have set aside \$20,000 to promote it further. Peer review is evidently of secondary interest when such financial manoeuvres are involved.

And so, to those who chafe under the pettifoggery and nitpicking annoyances of the comments of reviewers who "don't seem to understand how important this piece of research is", let us counsel patience, forbearance and cunning. Hang in there, and remember that, after all, if you can't convince the reviewer, you may not be able to persuade other readers. Also, you, too, may perhaps be a reviewer some day.

correspondence

Earthquake risk

SIR,—The succession of devastating earthquakes in recent months has vividly borne out O'Keefe *et al.*'s conclusion¹ that "natural" disasters are on the increase. More remains to be learned about precursory phenomena before earthquakes can be forecast reliably enough to save lives regularly. Yet much can be done in other ways to mitigate the hazards. Earthquake risk is highest where the probability of earthquake occurrence and the vulnerability of an area combine to maximum effect, and in practical terms it is this that requires quantitative evaluation. Molchan *et al.*² have developed a mathematical analysis of earthquake risk but whereas evaluation of earthquake occurrence has attracted considerable attention, through compilation of seismic zoning maps and so on, the evaluation of vulnerability has received much less. Vulnerability is the distribution of loss potential, measured in financial terms, and is given by the actuarial assessment of the damage potential in an area both of material losses and social losses (loss of life, injury, loss of work, family, social disruption).

Planning must attempt to reduce vulnerability in areas of high seismicity, but it would be impracticable to design for total invulnerability. Hence, a measure of degree of vulnerability is needed. Engineers use the concept of earthquake resistance in structural design. I propose a similar concept of earthquake resilience should be used in the same way to assess the social and economic response. Just as engineers accept that some degree of damage to structures in an earthquake must be tolerated, so it must be accepted that some social losses have to be faced. Certain essential services such as hospitals, and fire fighting units may need special protection, both in terms of buildings and personnel. But other structures and services may perhaps be disrupted without long term detriment to the society. The concept of resilience provides a means of estimating the power of recovery of society in the event of an earthquake. With this methodology, the balance can be worked out for an acceptable expenditure to provide a tolerable degree of protection against an earthquake, which can be afforded within the economy of the country as a whole and

which forms an integral part of its national development plan.

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¹ O'Keefe, P., Westgate, K. and Wisner, B. "Taking the naturalness out of natural disasters," *Nature*, 260, 566-567 (1976).

² Molchan, G. M., Keilis-Borok, V. I., and Vilkovich, G. S., "Seismicity and principal seismic effects," *Geophys. J. R. astr. Soc.*, 21, 323-335 (1970).

Significant journals of science

SIR,—The review article of Garfield (December 16, 1976, pages 609-615) contains relevant information in Table 3, where the impact factor (that is, the ratio between the citations in the 1974 Science Citation Index and articles published) of scientific journals is being presented in three specialities. Tables 1 and 2, on the other hand, are misleading. The ranking is by total number of citations. This criterion is invalid for a cross-section of all scientific journals.

In a field, say general medicine, where as a matter of example 10,000 workers each publish a paper with on average 10 references, this means 100,000 citations; material science, with 100 investigators and the same ratio, would yield only 1,000 citations. Thus the total number of citations, and in some lesser measure the "impact" too, are influenced by the total number of people interested in a given speciality.

Of the many factors influencing the number of laboratories working in any given field (besides fashion, inertia and others), last but not least, public funding must also be mentioned. The number of citations is rather a measure of the financial endowment of the field and not of the "significance" of the journal.

The 206 journals cover well established and thoroughly ploughed fields. Somewhat speciously it could be argued that for correlative, innovative, out-of-the-common and modern topics, which have not had time to accumulate citations and large teams, one must look outside of the 206.

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Rapid publication

SIR,—Rapid publication has become a topic of increasing importance as the camera-ready method and related methods have become more and more popular, especially for papers presented at symposia and the like. The manuscripts must be prepared by the authors according to special instructions. In small institutions the authors have to do all the processing themselves in addition to preparing the normal manuscript. They soon find out that a paper of 5-6 pages will take three to four weeks of drawing, photographing, editing, and typing according to the instructions. At a symposium with 200 to 250 papers, I would guess at least 100 will be prepared by the authors. This means that over 7 years are spent by scientists in preparing the camera-ready manuscript!

Institutions in developing countries usually tend to be small and have small budgets, and scientists working there are already overloaded with a wide variety of problems inherent in such situations. The preparation of camera-ready manuscripts therefore lessens the costly time which could be spent on actual research. Compared to scientists in well-equipped institutions, the scientists in small institutions see their research activities fall increasingly into arrears. I think that for us and other scientists in similar situations the advantages do not always equal the time and misery of preparing a camera-ready manuscript. I realise that the advantages for editors as well as for well-equipped scientists are quite substantial.

In case camera-ready manuscripts are required, I would suggest that possibilities for technical assistance are created in this respect for those who do not have it, and for which biologists are certainly not trained.

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He's repented

The most recent issue of *Scientia Sinica* contains the following correction slip: In the article "Devote Every Effort to Running Successfully Socialist Research Institutes of Science" (*Sci. Sin.*, XIX, No. 5), "the arch unrepentant capitalist-roader in the Party Teng Hsiao-ping" should read "Teng Hsiao-ping".

news and views

Immunological networks

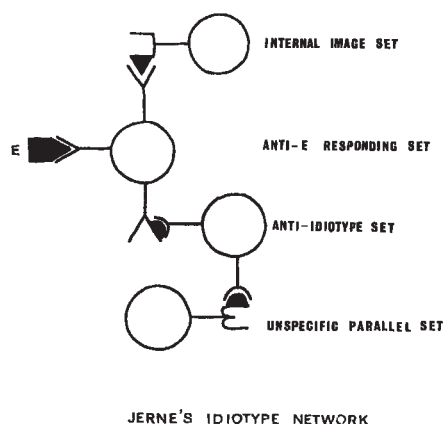
from Martin Raff

SEVERAL years ago, Jerne proposed a network theory of the immune system^{1,2} and predicted that, if correct, its impact on immunology could be as great as that of the selective theories of antibody formation³. Although crucial information is still lacking, increasing evidence suggests that he may be right.

Idiotypic networks

Antibodies not only recognise antigens, they themselves can act as antigens. Those antigenic determinants (or epitopes) that are related to the antigen-combining site of an antibody are called 'idiotypes'. Jerne suggests that idiotypes are more than just useful tools for immunologists—they may form the basis of a complex immunological network which is central to the functioning of the immune system. He argues as follows. Since the repertoire of antigen-binding sites (and thus idiotypes) in an animal is very large (perhaps $\sim 10^6$), no one idiotypic determinant, in the absence of antigenic stimulation, is present in high enough concentration to induce tolerance. Thus in the course of an immune reaction the increased concentration of specific idiotypes would stimulate anti-idiotypic immune responses. Such anti-idiotypic (and presumably, in turn, anti-anti-idiotypic) responses would play an important part in regulating an immune response. It is known that animals can be stimulated to make antibodies to the idiotypic determinants of antibody molecules produced by other animals of the same species or strain, or even of their own antibodies (see below); and this suggests that an animal possesses antigen-binding sites that can recognise any idiotypic occurring in the species. This in turn suggests that within an immune system, any idiotypic can be recognised by a set of antigen-binding sites and any antigen-binding site will recognise a set of idiotypes.

Thus, a lymphocyte clone, with Ig receptors having an antigen-binding



site for epitope E and bearing a characteristic set of idiotypic I, can in principle form a functional network with two other sets of lymphocytes (see diagram)—idiotypic-reactive cells with receptors having binding sites for I, and cells with Ig receptors bearing idiotypes which are cross reactive with E and thus recognised by the anti-E lymphocyte clone. Jerne calls the latter set the "internal image" set of lymphocytes. A fourth set of cells in this interacting network might consist of cells with Ig receptors bearing I idiotypic determinants but having antigen-binding sites for epitopes other than E. In addition, each of these three lymphocyte sets, which are functionally linked by idiotypic to the original anti-E lymphocyte clone, forms its own interacting network with other lymphocytes, also built around the idiotypes of their Ig receptors. Assuming that both T and B lymphocytes share at least some idiotypes³⁻⁵, both classes of lymphocyte would participate in such a functional network, which is in a dynamic steady state with the elements interacting both positively and negatively. Jerne tentatively proposes that the anti-idiotypic (anti-I) set of lymphocytes may have an inhibitory effect while the "internal image" set may have a stimulatory effect on the anti-E clone, the overall

effect being a "balanced suppression" which must be overcome in order to induce an immune response to E (ref. 2). However, it may be that within each set both stimulatory and suppressive lymphocyte subpopulations are represented.

The immune system is thus visualised as a complex network of antigen-binding sites that recognise sets of idiotypes, and of idiotypes that are recognised by sets of antigen-binding sites. Its response to antigen is, therefore, best understood in terms of a reverberating perturbation of the network, rather than as a response of individual antigen-sensitive lymphocytes.

Many of the elements required for such an idiotypic-based network have now been shown to exist. T (refs 6-8) and B (refs 8-17) lymphocytes reactive to idiotypes present on the animal's own Ig molecules have been unequivocally demonstrated. Anti-idiotypic antibodies, administered exogenously^{3-5,7,18-20} or produced endogenously^{16,17,21} have been shown to suppress^{3-5,7,18-19,21} or stimulate^{4,20} T and/or B lymphocytes which share the relevant idiotypes, and anti-idiotypic-reactive T cells have been shown to suppress the production of antibodies carrying the idiotypic⁷. Moreover, it has been demonstrated that the antibody response to phosphorylcholine in BALB/c mice, which is largely restricted to the response of a single clone carrying the TEPC 15 idiotypic¹⁸, is accompanied by the production of anti-idiotypic (anti-TEPC 15) antibodies^{13,15}. Therefore, idiotypic-bearing and complementary anti-idiotypic reactive lymphocytes coexist in the immune system and, in at least one case of a clonally restricted antibody response^{13,15}, both sets of lymphocytes are activated during an immune response to an appropriate antigen. What remains to be demonstrated is that such autogenous anti-idiotypic reactions occur in the more common situation where the initial response is

clonally heterogeneous, and that they have an important immunoregulatory role. *A priori* it seems highly likely that they do.

Moreover, it makes sense that just as environmental antigens stimulate and recruit relevant T and B cell clones into the recirculating, long-lived lymphocyte pool, the antibodies and receptor-bearing cells produced in the course of these responses should stimulate and recruit anti-idiotypic clones into the pool; thus, the immune system would organise itself into an idiotypic-based network. It should not be difficult to establish that such a network is important in immunoregulation: if eliminating anti-idiotypic-reactive T and B cells from a cell population markedly alters the response of the idiotypic-bearing clones to the appropriate antigen, the point would be made. From a practical standpoint, the TEPC 15 idiotypic response to phosphorylcholine^{14,15,16,18} would be an attractive system for such an experiment, but ultimately it will be important to demonstrate an effect in a non-restricted, clonally heterogeneous response.

As Jerne has pointed out², the major weakness in an immune network theory at the moment is the incompleteness of our knowledge of the component parts (immunologists are still subdividing T and B cells into an increasing number of subclasses, and the end is not yet in sight) and our ignorance of the mechanisms involved in positive and negative interactions between the parts. The task is clear, and it seems unlikely that mathematical model-building^{22,23} will be of great value until more information about the elements in the network is available. In the meantime, the lack of detail makes it possible to use the network theory to explain various immunological paradoxes that are difficult to explain in other ways³. For example, it provides, in principle, a way out of the Oudin-Cazenave enigma²⁴, which is the observation that antibodies produced against two non-cross-reacting epitopes of one antigen molecule may have similar idiotypic determinants. It can also explain another of Oudin's paradoxical observations²⁵—that immunisation results in the production of nonspecific Ig molecules bearing the same idiotypes as the specific antibodies; in fact, this suggests the existence of the "unspecific parallel set" of lymphocytes in Jerne's network (see Fig.).

An allotype network

The dramatic observations of the Herzenbergs and their colleagues²⁶ on chronic allotype suppression have uncovered another type of lymphocyte network, based on allotype rather

Volcano spacing in East Africa

from Peter J. Smith

THE near-rectilinear pattern of volcanoes in the Galapagos Islands was first noticed by Darwin (in *Geological Observations on Volcanic Islands*, London, 1891) and the near-uniform spacing of Hawaiian volcanoes was first observed by Green (in *Vestiges of the Molten Globe*, Honolulu, 1887). Since then, numerous workers have claimed to detect regularities in volcano siting, and some have pointed out that the spacings of oceanic volcanoes in particular appear to be roughly equal to the thickness of the local crust. More systematic attempts to relate volcano spacing to crustal thickness were doomed to failure, however, because (as we now know) the crust is not the correct unit to consider in this context.

Interest in the problem of volcanic intervals therefore largely died out until in 1974 Vogt (*Earth planet. Sci. Lett.* **21**, 235; 1974) revived it in the light of modern ideas on Earth structure. Vogt found that within oceanic provinces volcano spacings are indeed remarkably uniform. In 'continental' subduction zones volcanoes are typically 70 km apart whereas in island arcs they are 55 km apart. The separations of volcanoes associated with 'hot spots', on the other hand, increase with the age of the surrounding oceanic crust. But what, figuratively speaking, links all these volcanoes is the thickness not of the crust but of the lithosphere. Thus Vogt was able to show that within any given volcanic province the distance between adjacent volcanoes is roughly

equal to the thickness of the local lithosphere.

But is this also true for continental volcanism? To find out, Mohr and Wood (*Earth planet. Sci. Lett.* **33**, 126; 1976) have examined volcanoes along the northern section of the East African rift (roughly 15°N–5°S). This is a particularly suitable area because, although data are fewer, lithospheric thicknesses are generally better determined than in oceanic zones and are known to cover a much wider range. In any event, there are sufficient data to allow Mohr and Wood to show convincingly that the volcano spacing = lithospheric thickness relationship holds good for East Africa as well and is independent of the type of volcanic rock involved.

Why the relationship holds, apparently universally, is less clear, however. Vogt made two suggestions: first, that a volcano taps all magma within a given radius (a 'scavenging radius') related to lithospheric thickness through a conical volcanic conduit of fixed angle (25°) and, second, that volcanic sites are controlled by lithospheric fracture grids with spacings related to lithospheric thickness. He favoured the latter mechanism, though leaving open the question of whether the grids predate, or are contemporaneous with, the magmatic activity. By contrast, in a situation in which the crust is exposed Mohr and Wood find no evidence at all of volcanic control by pre-existing fracture grids, although again the possibility of contemporaneous rifting remains open.

than idiotypic. Allotypes are alloantigenic epitopes on Ig molecules which are unrelated to the antigen binding site and have no known functional significance. In [SJL(Ig-1b) × BALB/c (Ig-1a)]F₁ mice, perinatal exposure to antibodies directed against Ig-1b allotype determinants (present on IgG molecules) results in the generation of suppressor T cells which specifically inhibit the production of all antibodies bearing the Ig-1b allotype but not antibodies bearing the other parental allotype (Ig-1a). Herzenberg *et al.*²⁶ have now shown that the suppressor T cells in these allotype-suppressed mice do not directly act on Ig-1b B cells, as originally assumed; rather, they suppress a specific class of helper T cells which normally help Ig-1b B cells to differentiate into Ig-1b antibody-secreting cells. Besides indicating that T cell suppression of B cell responses

may act indirectly by way of T helper cells, as is probably also the case in carrier-specific T cell suppression²⁷, these striking experiments demonstrate that, in these mice at least, there are allotype-specific T helper cells, which help only B cells bearing the Ig-1b allotype, and suppressor T cells which specifically suppress these allotype-specific T helper cells. These three sets of cells constitute a lymphocyte network organised around allotype, which, unlike an idiotypic-based network, is independent of antigen specificity and would therefore include many lymphocyte sets functionally linked in an idiotypic network.

There are however difficulties in inducing chronic allotype suppression using other mouse strain combinations²⁸, and it is not clear whether allotype networks are the exception or the rule. Nor is it obvious what pur-

pose they serve. Since it is not known whether allotypes have any functional significance one cannot assess the value of regulating immune responses on the basis of allotype. On the other hand, there would be obvious advantages in regulating immune responses on the basis of Ig class. At the moment, the only hint of lymphocyte networks based on Ig class is the report of T helper cells that are specific for IgE B cells²⁹. It is possible that the finding of an allotype-based network will point the way to an eventual demonstration of similar lymphocyte networks based on Ig class. In view of the increasing evidence for Ia-bearing molecules with immunoregulatory function, and Ia-bearing cell-surface acceptors for these molecules (see ref. 30), it is also conceivable that there may be lymphocyte networks organised around determinants of the major histocompatibility complex (MHC), just as there are around Ig epitopes.

There are other intriguing questions raised by the demonstration of an allotype-based network²⁶. How do the cells in the network recognise each other? The conservative answer would be by the use of allotype-bearing and allotype-recognising antibody molecules. But it has been difficult to demonstrate Ig allotypes on T cells; and the relatively high concentrations of allotype in the serum might be expected to block allotype-reactive receptors on cells. What is the relationship between carrier-reactive and allotype-reactive T helper cells? Apparently, both can be required to activate a B cell, but are they the

same cell, with one receptor for allotype and one for carrier? Since evidence is growing that most T cells preferentially respond to antigen in association with MHC determinants (see ref. 30), is it possible that helper T cells have separate receptors for carrier, allotype (and/or Ig class) and MHC antigens? □

- ¹ Jerne, N. K. in *Ontogeny of Acquired Immunity* (ed. Porter, R. & Knight, J.), Ciba Foundation Symposium New Series 5, 1 (Associated Scientific Publishers, Amsterdam, 1972).
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Incident radiation returns to the receiver from the tissue both by reflection from large surfaces and reradiation by any small elastic particles in the beam path. Donald (*Brit. J. Radiol.* **49**, 306; 1976) has recently discussed the use of reflected radiation from structures of differing acoustic impedance and Gosling (*IEE Medical Electronics Monographs* **21**, 73; 1976) has considered the use that can be made of the backscattered Doppler shift signals from moving blood in arteries.

When a blood vessel is bathed in an ultrasonic beam a wide spectrum of Doppler-shifted frequencies is back-scattered from vessel to receiver at each instant of time, because velocities of the red cells vary across the lumen. This spectrum varies over the cardiac cycle and, with beam frequencies of 1 to 10 MHz, is in the audio range for both healthy and diseased arteries. All signals received from a vessel can be displayed as a sonagram—the Doppler shifted frequencies along the Y axis, time along X and blackness of trace proportional to signal amplitude. The striking aspect of sonagrams from any vessel is their well-defined maximum frequency envelope over the cardiac cycle which can be used to characterise an arterial site under specified conditions.

Although certain direct physiological information can be obtained from a single sonagram a great deal more information can be gained by using two simultaneous observation sites at consecutively paired positions along the vascular tree. Phasic changes in such paired sonagrams are easily recognised as 'normal' or abnormal. These changes may be due to trauma, congenital defect or pathological change and the observed differences from normal may be correlated empirically with clinical condition and used diagnostically.

As well as arterial status, functional ability of the heart can be assessed. This is done with one Doppler detector over a neck artery, a second on the chest plus an ECG and a heart microphone. A simultaneous four line trace shows all the cardiac intervals (Gosling, Beard and Pickup, in preparation).

No Doppler shift ultrasound volume flowmeter is yet available for clinical use. However, it has been realised that the shape of the flow-velocity pulse at any arterial site, and hence the flow-velocity sonagram, carries information about the whole cardiovascular tree. Baskett *et al.* (*Cardiovascular Res.* 1977 in the press) has used this approach to estimate the extent of disease in the internal carotid artery supplying the brain (a common source of strokes). An intrinsic property of an artery wall is its compliance. This is defined as fractional change of

Ultrasonic assessment of arterial pathways

from C. D. Field

Non-invasive determination of blood vessel function by measurement of Doppler shifted ultrasound reflected from moving red cells is rapidly gaining clinical acceptance as a useful tool in screening for vascular disease. Abnormal patterns in the reflected Doppler-shifted signals compared with those obtained from normal healthy individuals are beginning to be characterised and correlated with various clinical conditions, as defined by conventional radiography and other techniques, and to be used diagnostically. For routine screening, ultrasound has several important advantages over conventional radiography. Injections of toxic dyes are not needed to locate the

vessel under study, and because power levels are so low (about 30 mW cm⁻²) measurements can be repeated as often as required.

To detect red cell velocities by Doppler shifted ultrasound two identical piezoelectric crystals are mounted coplanar at an angle to the vessel under study, as transmitter and receiver. Activation of the transmitting crystal produces a narrow beam of ultrasound which is coupled into the tissue by way of a water-based gel. Activation frequencies vary from 1 to 10 MHz and their physical properties are well documented (Gosling and King, *Cardiovascular Applications of Ultrasound*, North Holland, 1973).

lumen diameter per 10 mmHg pressure and so may be compared in subjects with differing pulse pressures. Gosling (*op. cit.*) considers that it may be a useful indication of vascular disease and that *in vivo* measurement of this parameter could lead to earlier detection of abnormalities. There is some difficulty in measuring compliance directly but it has been demonstrated that in certain conditions the average value of compliance for any length of artery is given by a simple function of the delay time between entry and exit pulse waves, that is, sonagrams, for the same heart beat and their distance apart on the vessel. This indirect measure of compliance is easily made through the skin, and amounts to determination of the apparent propagation speed of the flow velocity pulse.

The Non-Invasive Angiology Group at Guy's Hospital Medical School, London, has recently applied this pulse wave velocity approach in a study of compliance in aorta, pelvic arteries and leg arteries. They find that aortic compliance in normal subjects increases by some 70% in the first decade for both males and females, decreases to birth value over the next decade and then only slowly decreases over the rest of the life span to about half-birth values in old age. Their results also show that the female diverges from the male at menarche. Women retain a significantly higher aortic compliance until menopause, after which there is again no difference. In patients with diabetes it was found that insulin-dependent juveniles (diagnosed less than one year before measurement) had abnormally high aortic compliance, but that older patients (over 40 years) whose diabetes was controllable by diet exhibited normal or lower values. These results are markedly different from the accepted view that diabetic children and adults always have stiffer arteries than normal. That physiological function may affect structure has recently been demonstrated by Berry *et al.* (*Brit. Heart. J.* **38**, 510; 1976) who showed that compliance of the aortic-iliac pathway is anomalously high in those children born with a single umbilical artery. Such compliance studies as these clearly have implications in the search for causal factors of arterial occlusive disease, the biggest single cause of death in society today.

It may be concluded that Doppler-shifted signals from various arterial sites and pathways of the body can yield much useful physiological and pathological information. Research teams such as those at Guy's Hospital are now able to scan, non-invasively, specific arterial segments (or pathways) and objectively decide whether they are

normal or not. Furthermore, this is being done on an out-patient basis in the relaxed unsedated patient. Although still in their infancy as clinical tools, these techniques and methodology offer a complementary approach to conventional methods with obvious advantages to clinicians and patients alike. □

Diamond platelets are interstitial carbon

from John Walker

IN a recent *News and Views* item (*Nature*, **264**, 317; 1976) I noted in passing that we were still ignorant of the nature of one of the defects found in diamond—the so-called 'platelets'. This ignorance has now been rectified by G. S. Woods (*Phil. Mag.* **34**, 993–1012; 1976). The platelets (large planar defects on {100} planes) are apparently composed of carbon atoms in interstitial positions (that is, squeezed into the empty interstices between the normal lattice sites).

An anomaly in the X-ray diffraction photographs of some diamonds was first noted by Raman and Nilakantan in 1940. The normal diffraction photograph contains a symmetrical array of spots corresponding to reflection of the X-rays from particular planes of atoms. But in some diamonds—what we now

call type Ia—the spots were accompanied by streaks ('spikes'). In 1956 Frank (*Proc. R. Soc. Lond.*, **A237**, 168; 1956) proposed that the streaks were due to planar aggregates of foreign atoms, oriented along the {100} planes—the platelets. His prediction was confirmed by Evans and Phaal in 1962 (*Proc. R. Soc. Lond.* **A270**, 538) when they observed the platelets directly using electron microscopy.

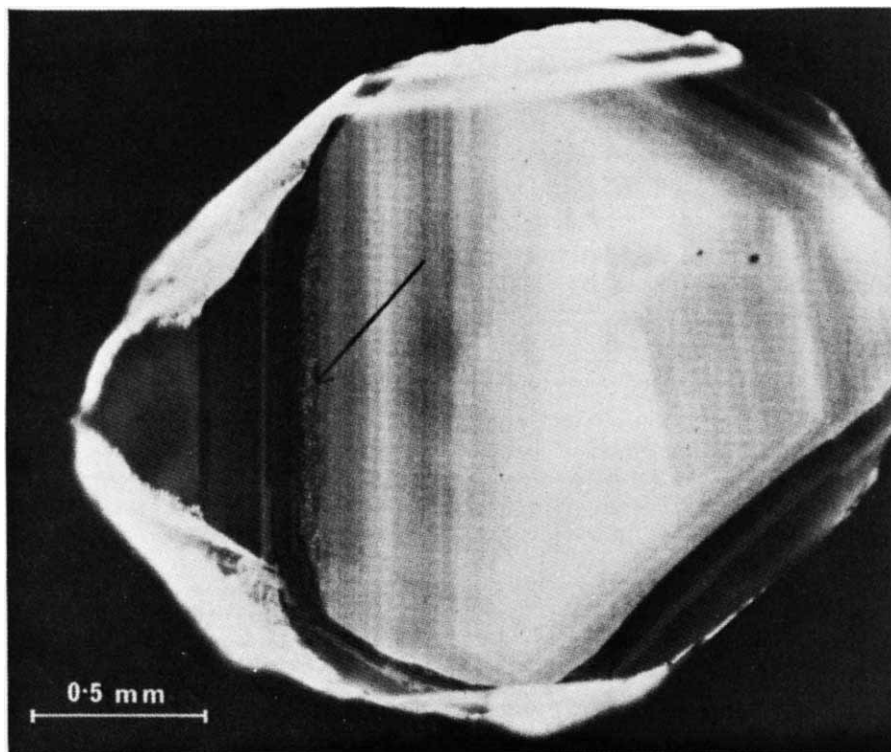
Frank had proposed that the foreign atoms were silicon. Subsequently analyses showed that this element was an uncommon impurity in diamond, whereas concentrations up to ~12% were required to account for the platelets. When Kaiser and Bond (*Phys. Rev.* **115**, 857; 1959) showed that type Ia diamonds contained nitrogen in the required concentration, the obvious conclusion was that the platelets were made of nitrogen—obvious, but wrong.

In 1969 Sobolev, Lisoivan and Lenskaya (*Sov. Phys. Dokl.* **12**, 665) showed that the platelet concentration, as measured by the X-ray spike intensity, did not correlate with the total nitrogen concentration as measured by the infrared absorption peak at 7.8 μm . The platelets could contain little if any nitrogen.

These results, though controversial, were soon confirmed by a completely independent technique—thermal conductivity. The way the thermal conductivity of a crystal varies with temperature depends on the size and concentration of any defects in it. Turk and Klemens (*Phys. Rev.* **B9**, 4422,

Cathodoluminescence pattern from a diamond. The 'giant' platelets are

individually resolvable as white dots in the arrowed band.



1974) showed by this method that at least 97% of the nitrogen in the diamonds they studied was present in aggregates smaller than one nanometre in diameter. No more than 5% could be in the platelets, which are typically 50 nm across.

So what could the platelets be made of? Diamonds had been analysed by many different techniques and no other impurity had been found in anything like the proportions necessary. Evans (*Diamond Res.* page 6, 1973) therefore proposed that they were carbon atoms; but carbon atoms arranged in such a way as to break the normal periodic stacking sequence of the diamond lattice—a plane of interstitial carbon atoms. Woods's paper confirms Evans's hypothesis.

Woods managed to find diamonds containing 'giant' platelets up to 5,000 nm in diameter (see diagram) which he then studied by electron microscopy. He confirmed that these were just large examples of the normal platelets by observing in one of his specimens a continuous gradation in size, down to the more usual 50 nm. Accompanying the giant platelets were loops of perfect dislocation, of interstitial character (like the platelets), and also lying in {100} planes. Their shapes, dimensions and geometries were so unusual, and so similar to the platelets, that Woods concluded they were produced by the platelets. (The alternative possibility, that they produced the platelets, seemed unlikely because the platelets were sometimes present when the loops were not, whereas the converse was never observed). Some of the dislocation loops were L-shaped—they changed from one to another non-parallel {100} plane. Woods therefore proposed that they were created by the interaction of two perpendicular platelets. When the two grew large enough to touch, or came sufficiently close to feel each others' strain fields, two dislocation loops would be created (or a single L-shaped loop if the two loops were in contact). The crucially important point is that, given the geometry of the platelets, of the loops, and of the diamond lattice, and bearing in mind that the platelets are interstitial in character, the platelets can create the loops only if the platelets themselves are composed of carbon atoms in interstitial sites.

If Woods's interpretation is correct—and it is certainly consistent with all the extant data—then he has solved a puzzle that has fascinated diamond physicists for years.

As usual, however, there are questions that are still unanswered. Why is it that platelets are seen only in diamonds containing nitrogen aggregates? Are the platelets formed by

interstitial nitrogen atoms displacing substitutional carbon, as Evans suggested? How exactly do the platelet atoms bond to the rest of the lattice? Why are platelets never seen in synthetic diamonds containing nitrogen? Do the platelets play any part in diamond synthesis, or are they merely the product of ageing in diamonds containing nitrogen? Woods's paper is likely to stimulate new thinking on all these questions. □

Exploiting exotic plants

from Robert M. May

ALTHOUGH more than 3,000 plant species have been utilised by man for food, only about 20 major crops today provide food for most of the world.

Motivated by the thought that "to help feed, clothe, and house a rapidly increasing world population, it is timely to consider neglected or little-known plant species", a panel of the National Research Council of the US National Academy of Sciences has presented a report on *Underexploited Tropical Plants with Promising Economic Value* (published by the Board on Science and Technology for International Development, NRC, 1975: see also the excellent summary by Langford, *NAS News Report*, 26 (8), 1; 1976).

The report begins by noting that, in tropical countries, concentration on a small number of major food crops stems not from their inherent superiority, but rather from historical accident. "Many indigenous species may possess equal merit, but were disregarded during the colonial era when consumer demands in European countries largely determined the cultivation (and research) priorities in tropical agriculture". In addition, "most agricultural scientists are unaware of the scope and potential offered by tropical botany. The discipline suffers largely because the major centres of scientific research are located in temperate zones" (NRC, *op. cit.*).

Reliance on a small number of plant species carries risk, because monocultures are vulnerable to catastrophic failure brought about by disease or climatic fluctuations. But, due to the sprawl of cities, the high cost of maintaining botanic gardens and habitat reserves, and the spread of conventional agriculture, potentially useful plants (including varieties necessary for genetic diversity in the development of new food crops) are being destroyed,

and some are "in imminent danger of extinction". In urgently recommending programmes of systematic collection and study of tropical flora, the NRC panel hopes that many under-exploited plants may thereby follow the example set by the soybean, which has become a staple in many parts of the world only within the past 50 years.

The report describes 36 plants, selected from among 400 nominated by plant experts in many countries. For each of these 36, the report indicates the advantages and limitations of the plant, and outlines special requirements and research needs.

A prime example is quinoa. This tall, hardy herb of the Andean altiplano was a staple in the Inca empire. Its grain is rich in protein, with a good balance of amino acids; it is possibly a better protein source than most of the true cereals. Its disadvantage is a bitter taste, derived from saponins in the outer seed layer. The sort of questions that arise for quinoa are fairly typical: can it be grown at lower altitudes and in warmer climates; can a satisfactorily saponin-free variety be produced; or should the saponin be retained as a natural defence against pests, and be milled out after harvesting?

A native palm of the Amazon region, *Jessenia polycarpa*, bears fruit clusters from which can be extracted a food oil akin to olive oil. None of the several *Jessenia* species has been cultivated commercially. To quote Langford, "that no one knows how many years are required for *Jessenia* to mature and bear seed indicates the extent of research needs pertaining to many tropical plants".

Naranjilla, "the golden fruit of the Andes", is little known outside Colombia and Ecuador, where it is used to flavour confections and preserves, and to make a popular fruit drink. The plant could apparently flourish, and be put to these uses, throughout the African and Asian tropics.

In Chile's salt-covered Atacama desert, the leguminous plant tamarugo not merely survives, but can sustain grazing sheep at a density approaching that for green pasture. The report observes that since 1968 more than 5,000 sheep have been raised on tamarugo plantations, and recommends that trials be undertaken in other parts of Latin America, the Middle East, and in arid Africa.

Turning from food and forage crops, the report discusses, for example, guayule. This desert shrub, found in Mexico and the southwestern USA, produces a latex which, when refined, is similar to the rubber obtained from *Hevea* trees in southeast Asia.

The report emphasises that all such

novel plants should be seen as complements to, not as substitutes for, conventional tropical crops. It also acknowledges that the problems of exploiting new plants are not just horticultural, but are economic and sociological: new crops depend on the creation of new markets. □

Nucleon coalescence

from P. E. Hodgson

RECENT experiments on the interaction of relativistic heavy ions with silver and uranium have provided evidence that the emitted nucleons tend to stick together to form composite particles, so that the numbers of high energy ${}^3\text{He}$ and ${}^4\text{He}$ particles are two or three orders of magnitude greater than those found for proton-induced reactions at comparable velocities. It was found that more ${}^3\text{He}$ were emitted than ${}^4\text{He}$, and this made it doubtful whether both their emission could be explained by the same process.

To study this matter in more detail, Gutbrod and colleagues (*Phys. Rev. Lett.*, **37**, 667; 1976) have measured the energy spectra at several angles of the

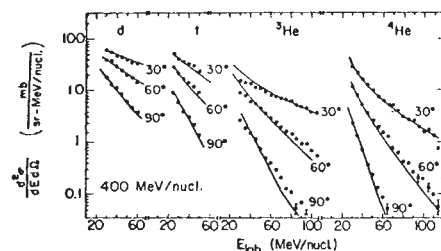
protons, deuterons, tritons, helions (${}^3\text{He}$) and alpha particles (${}^4\text{He}$) emitted when uranium is bombarded with 250, 400 and 2,100 MeV per nucleon ${}^{20}\text{Ne}$ ions and with 400 MeV per nucleon alpha particles, and some typical results are shown in the figure.

To try to account for these distributions, they developed a theory that had already been used by Butler and Pearson to explain the observation of high energy deuterons emitted from nuclei bombarded with energetic protons. This theory assumes that among the cascade of nucleons emitted from such reactions there will be some neutron-proton pairs with rather low relative momenta. The nucleons of each pair can interact with each other and with the surrounding nuclear field serving to absorb excess energy and momenta. This model enables the energy spectra of the deuterons to be calculated from that of the cascade nucleons.

The theory was modified to calculate the spectra of the lighter particles emitted from relativistic heavy ion collisions, and the results are compared in the figure with the experimental data. It is seen that the overall features are accounted for very well.

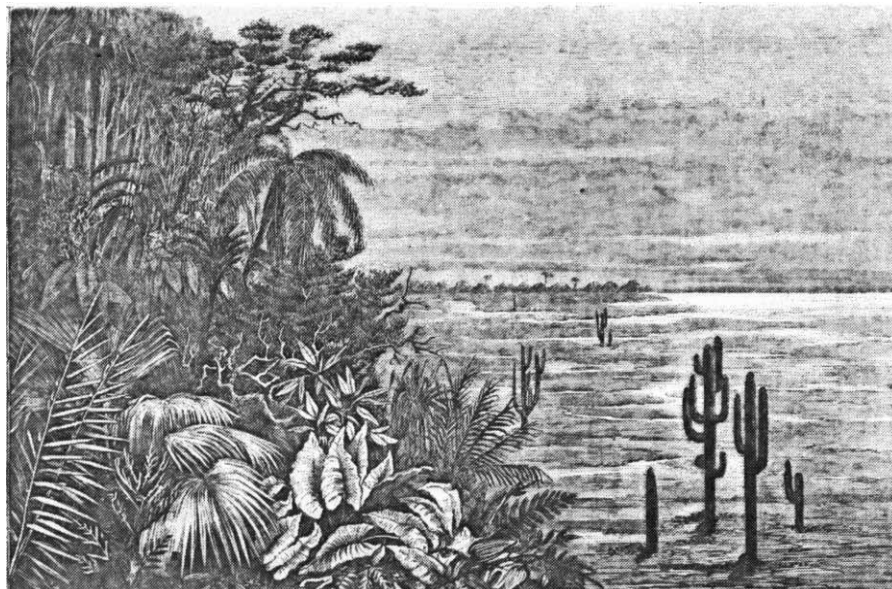
An important parameter of the theory is the greatest relative momentum between the neutron and the proton for which they can coalesce. This momentum was adjusted for each of the reactions investigated so as to give the best overall fit to the experimental data, and it was found that the best values were around 130 MeV/c in each case. This constancy checks the validity of the theory.

It still remains to understand the details of the mechanism of nucleon coalescence, but it is certainly encouraging that a relatively simple theory can account so well for so much experimental data relating to very complicated reactions.



Experimental energy spectra of the deuterons (d), tritons (t), helions (${}^3\text{He}$) and alpha particles (${}^4\text{He}$) emitted from uranium irradiated by ${}^{20}\text{Ne}$ ions at 400 MeV per nucleon.

A hundred years ago



TROPICAL FORESTS IN HAMPSHIRE. The Valley of the Bourne restored to represent the conditions which are supposed to have existed during the deposition of the Lower Bagshot Formation: Looking at the scene from

a southern standpoint we should see to the north the distant chalk range. Whilst along the shore of the opposite bank of the valley we could with some difficulty detect the various forms of vegetation, which we should see with

greater clearness in the more immediate foreground. In this valley a singular stillness must have prevailed, as no trace of animal life whatever has been found, except a feather and a few insect wings blown in from the southern bank.

Of the following at least we are pretty sure, and of numerous others we can be almost sure, but there are indications of very many besides, the relationships of which are at present but imperfectly defined.

Here we should see the graceful fan-palm and the feather palms, adding softness to the view by their elegantly-curved and drooping leaves, laurel and dwarfed oak, stately beeches, clumps of feathery acacia, trellised and festooned with smilax, the trailing aroid, with its large and glossy foliage and an undergrowth of *Mimosa* and of cypress in the swamplier ground, and variations in colour caused by the foliage of cinnamon and fig, and the ground clothed with ferns and sedges. On the barren sands of the distant valley are growing clumps of giant and weird-looking cactus. It is not difficult to picture to ourselves the view.

All this beauty is gone. We have nothing but these records of what must have been a view of great loveliness, which only the toil of the geologist can even faintly reproduce.

From *Nature*, **15**, January 18, 261; 1877.

review article

Homoeotic genes, compartments and cell determination in *Drosophila*

Gines Morata† & Peter A. Lawrence

Normal development of Drosophila depends on the activity of certain 'master' genes. We review recent work which suggests that these genes control the developmental pathways taken by specific groups of cells as they divide and construct precisely defined parts of the adult fly.

EVERYDAY experience tells us that the form of an adult animal is defined in great detail by its genotype. An example is an individual human face which, although each half develops separately, displays almost perfect bilateral symmetry. Yet how genes determine detailed structure, shape and size is unknown. We know more about the genetics of *Drosophila* than any other multicellular animal and yet even here we do not understand how genes control the development of any organ. If we want to unravel the genetic logic of development we could start with *Drosophila* and try to classify genes into those with a controlling role and those with a more subsidiary executive function.

The best candidates for controlling elements are the so-called homoeotic genes. Mutations in these genes characteristically transform one body part into another. They are particularly well studied in *Drosophila* but they have been found in some other insects^{1,2}. In essence the homoeotic transformations are replacements of normal structures by other normal structures which in wild-type flies are found elsewhere. The mutant *bithorax* is a good example of a homoeotic transformation; wildtype flies have a pair of wings and a pair of halteres (Fig. 1) but mutations in the *bithorax* gene³ transform the anterior half of the haltere into anterior wing. The wing tissue formed in the haltere in these mutant flies is very similar in all respects to normal wing tissue. The wild-type function specified by the *bithorax* gene is thus required during growth of the anterior cells of the haltere; if the function fails these cells will develop as in a wing. The mere existence of such a mutation indicates that there is at least one moment in normal development of the haltere in which the alternative—haltere or wing—is open. In this feature lies the current interest in homoeotic genes; they are single loci which determine the developmental pathways taken by groups of cells.

It has recently been discovered, in *Drosophila*, that special groups of cells are set aside at early stages of development, and that the cellular descendants of these groups form specific regions in the adult called 'compartments'^{4,5}. In this review we discuss the evidence that the development of compartments is under the control of homoeotic genes, and highlight the relationship between this new hypothesis⁴, and the older concept of cell determination. Since one

might expect similar types of genetic control of development in different groups of multicellular animals, we also briefly consider whether these phenomena occur in vertebrates.

In *Drosophila* the cuticular parts of the adult fly are constructed during the pupal period, from a set of 'imaginal disks' which form in the embryo and grow during the three larval stages: for example the wing imaginal disk forms the wing itself as well as those dorsal thoracic parts belonging to the wing segment. Most of the homoeotic mutants produce intersegmental transformations, the structures formed normally being found in another disk. Thus the *bithorax* mutation transforms the haltere segment into part of the wing segment (Fig. 1), *spineless-aristapedia* transforms the antenna into leg (Fig. 1), and *ophthalmoptera*, the eye into wing. There are at least two mutants that produce intrasegmental transformations where one part of the disk is the duplication of a different part of the same disk: the mutation *engrailed* transforms the posterior structures of wings into the corresponding anterior structures⁶ (Fig. 1). In *wingless* flies the wings are replaced by a mirror image duplication of the dorsal thorax^{7,8}.

In those cases where homoeotic mutations affect more than one disk they produce homologous transformations in different disks. For example, in *engrailed* posterior wing is transformed into anterior wing, posterior leg into anterior leg, and, probably, posterior haltere into anterior haltere. *bithorax* transforms not only the anterior haltere into anterior wing, but also, the anterior parts of the legs of the haltere segment into the anterior parts of the legs of the wing segment. Clearly there is a common requirement for the wild-type product of the *bithorax* gene in these two regions of the haltere segment, even though the cuticular structures produced by these two groups of cells are distinct. By contrast the type of cuticle in the anterior haltere is almost indistinguishable from that of the posterior haltere, yet the anterior disk cells which form the haltere are sensitive to the *bithorax* mutation while the posterior disk cells are not. Thus the function of homoeotic genes seems to be localised to particular regions of the body.

We need to define as exactly as possible the realms of action of the homoeotic genes. Unfortunately most mutations produce transformations with variable penetrance (penetrance=percentage of the mutant flies which show the phenotypic trait) and variable expressivity (expressivity=degree to which the mutant phenotype is shown by a given fly) which makes it difficult to define the limit of action of the wild-type genes. Moreover, the expressivity

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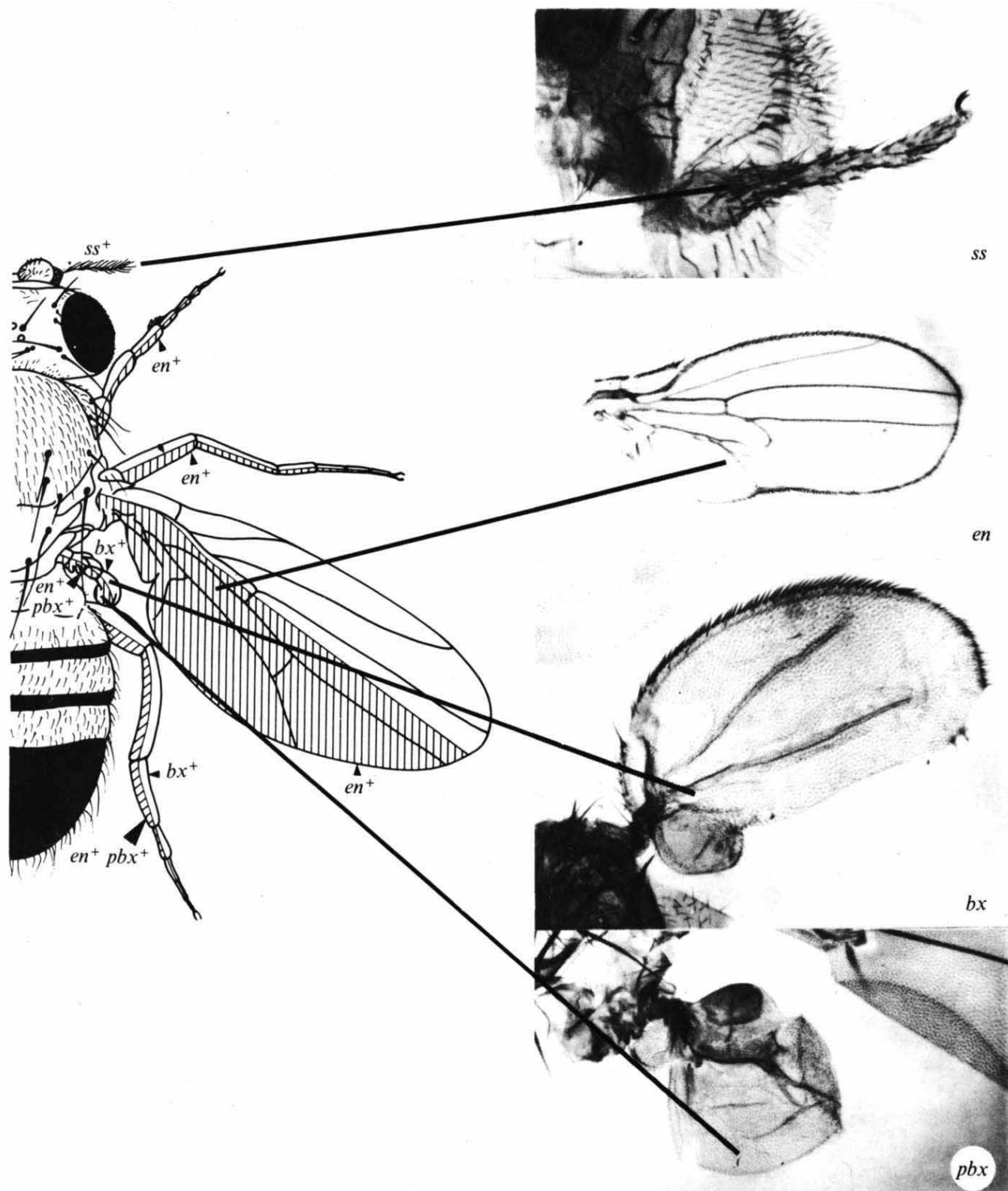


Fig 1. Homoeotic transformations produced by the mutants *spineless-aristapedia* (*ss*), *engrailed* (*en*), *bithorax* (*bx*) and *postbithorax* (*pbx*). A diagram of a wild-type fly is on the left. On the right there are photographs of some of the transformations produced by these mutants. *ss*: The antenna is transformed into leg. Mutations in this locus produce an effect in the antenna only. *en*: the posterior wing is partially transformed into anterior wing as witnessed by the effects on the veins^{6,17} and the posterior row of "anterior" bristles. This mutation also transforms the posterior legs into anterior and, probably, posterior haltere into anterior haltere. Consequently the activity of *en*⁺ is required in the posterior regions of at least these structures (shaded in the diagram of the wild-type fly). *bx*: the anterior haltere is transformed into anterior wing and also the anterior III leg is transformed into anterior II leg (this transformation can be recognised because the II and III legs are not identical, each having distinctive cuticular markers). The genotype of the transformation shown in the picture is *bx*³/*Df*(3*R*) *bxd*¹⁰⁰ where *Df*(3*R*) *bxd*¹⁰⁰ is a chromosome in which the *bithorax* gene is deleted¹⁵. For this reason the phenotype is very extreme. Notice the sharp boundary between the transformed anterior wing and the unaffected posterior haltere. Only the anterior haltere and anterior III leg require the activity of *bx*⁺. *pbx*: The posterior haltere is transformed into posterior wing and also the posterior III leg is transformed into posterior II leg (as in the *bx* transformation this can also be recognised by cuticular markers). The genotype of the transformation shown in the picture is *pbx*/*Dp*(3;3) *bxd*¹⁰⁰ *Df*(3*R*) *P115* where *Dp*(3;3) *bxd*¹⁰⁰ *Df*(3*R*) *P115* is a chromosome in which the *postbithorax* gene is deleted¹⁵. For this reason the phenotype is very extreme. Notice again the sharp boundary between the unaffected anterior haltere and the transformed posterior wing. Only the posterior haltere and the posterior III leg require the activity of *pbx*⁺.

of some of those mutants varies greatly in different regions of the same disk⁹. The probable reason for these incomplete transformations is that the mutants are leaky (*hypomorphs* in the *Drosophila* terminology) in which some wild-type function remains. Mutants like *Antennapedia* of *Rappaport* (*Ant^R*) produce a partial transformation of arista and antenna into leg so that the final structure is a mixture of phenotypically wild-type (arista or antenna) and mutant (leg) cells¹⁰. This is also the case for other mutants such as the different alleles of *spineless-aristapedia*¹¹ and *ophthalmoptera*¹² and some *bithorax* alleles. According to the description of *Antennapedia*¹⁰, it only affects the antenna part of the eye-antenna disk (even some antennal disk derivatives like rostralthaut and maxillary palpus are not affected) whereas *Nasobemia* which is probably in the same locus¹³ very often transforms part of the head cuticle belonging to the eye part of the disk¹⁴. It is conceivable that a very extreme *Antennapedia* mutation could transform the eye and perhaps other structures as well. For these reasons all homoeotic genes should ideally be studied with mutations that completely lack wildtype function—for instance with chromosomes that are deficient for the gene itself. Unfortunately this is usually impractical; the deficiencies are nearly always lethal when homozygous (we know that most deficiencies, even the smallest ones, are lethal¹⁵) and one has to be satisfied with the viable mutants that are most extreme. Even this is difficult, because too few mutant alleles are available (for example, there is only one allele of *engrailed*)—perhaps because homoeotic genes frequently mutate to a lethal form. For *bithorax*, where there are many alleles, a detailed genetic analysis has been done by Lewis^{3,16,17}. The mutation *bx³* is the most extreme known¹⁶; homozygous *bx³/bx³* flies show a phenotype only slightly less extreme than *bx³/bx⁻* (where *bx⁻* is the deficiency of the gene) which indicates that *bx³* almost completely lacks function of the *bithorax* gene. Thus in this case we can be reasonably certain that the extent of the phenotype *bx³/bx⁻* defines the domain affected by the *bithorax* gene.

We clearly need much more genetic analysis of homoeotic mutants, and new methods are now being developed: although some 90% of induced mutations in *Drosophila* are lethal¹⁸, only about 20% of these are cell lethal¹⁹—the phenotype of those lethal mutants that are viable in individual cells can be studied in clones made homozygous for the mutation²⁰.

Determination in mature disks

The concept of determination in the imaginal disks of *Drosophila* has been developed by Hadorn and his associates²¹ and discussed in detail elsewhere²². It refers to the acquisition by a group of cells of a commitment that precisely defines the fate of their descendants. Unlike many other developmental systems, the imaginal disks of *Drosophila* show a clear distinction between determined and differentiated state; one can demonstrate that the imaginal disks are determined to secrete certain regions of the adult cuticle much before this differentiation starts. An imaginal disk or a fragment of imaginal disk is considered to be determined if, when transplanted into the abdomen of a host larva, it differentiates autonomously into the same structures as *in situ*. When this type of experiment is performed on the mature imaginal disks it is found that they are strictly determined; the wing disk only and always forms wing structures, the first leg disk only forms first leg structures and so on. Their state of determination is more restricted than just leg or wing; within one disk, particular regions, when explanted, form specific parts of the adult structures, and in this way consistent fate maps of the mature disks have been constructed^{23,24} and in some cases even individual bristles have been located precisely in the disk²⁵.

The acquisition of a given state of determination seems to correlate with the ability of determined cells to recognise, and associate with, like cells and dissociate from unlike cells²⁶. Cells from a given disk or disk-fragment, after dissociation and mixing, remain mixed together and form integrated patterns of differentiated structures. However, cells from different disks or different regions of the same disk will sort out from each other and form separate patterns. In these experiments each type of cell differentiates autonomously according to its disk of origin^{26,27}.

We have no direct criterion for determination—the term is a purely operational one; determination may be stable in some experimental conditions but unstable in others. Thus, if imaginal disks are cut and the fragments allowed to grow in *in vivo* culture for several days or more, the state of determination can change. For example, groups of cells²⁸ in growing fragments of leg disks will frequently 'transdetermine' to other cuticular structures such as wing^{25,29}. Even the stability of determination of a given fragment depends on the precise experimental conditions: thoracic fragments of the wing disk will only make thorax after extra growth, but if mixed with wing fragments they make both thorax and wings³⁰. For these reasons experiments involving a long period of extra growth in *in vivo* culture cannot be used to deduce the state of determination that the initial fragment had *in situ*.

Determination in development

Determination probably begins at around the blastoderm period; if blastoderm cells are dissociated and mixed they will reaggregate specifically with cells that come from the same region of the egg³¹. If individual anterior blastoderm cells are transplanted to the posterior pole of the egg, they will not integrate with the foreign blastoderm cells, and will differentiate anterior structures in the adult³².

Clonal analysis is a powerful technique which has been applied to early development and can help to pinpoint the establishment of determination. Use is made of cell marker mutants which alter the colour of the cuticle or the shape of bristles in the adult fly but apparently do not affect the course of development. Staged eggs or larvae heterozygous for such a mutant are irradiated with X-rays. At low frequency, somatic recombination occurs in a cell so that one of its daughters becomes homozygous for the mutant, and generates a clone of cells of different genotype. After metamorphosis this clone is seen as a patch of marked cuticle on the adult. This method provides another criterion for determination, if mainly a negative one; when two structures are included in the same clone it shows that the original marked cell was not determined exclusively for either²². However, if two structures are never included in the same clone, it does not necessarily mean that their ancestor cells were determined differently. One must then show that the clones are large enough for at least some of them to have included the two structures and also that no physical barrier separates those structures in normal development.

In a careful analysis of early development of imaginal disks, it has been shown that clones produced by irradiation of the embryo at the time of cellular blastoderm can comprise structures of wing and second leg (both belong to the mesothoracic segment) proving that at that time the cells are not exclusively determined for either^{33–35}. Clones do not cross from one segment to another, suggesting that the segments are determined^{33–35}. Clones produced a few hours later were restricted to either second leg or wing³³. These results certainly indicate the appearance of a developmental segregation between ancestral cells for wing and leg in early development. Indeed, histological studies in another dipteran, *Dacus*, have shown that there is an initial group of cells of the mesothoracic segment that later become subdivided into dorsal (wing) and ventral (leg) imaginal disks³⁶.

Other evidence suggesting early determination comes from

experiments in which homoeotic transformations are produced by chemical or heat treatment^{37,38}; such changes are called phenocopies. For example, when early embryos are treated with ether as many as 30% of the flies show *bithorax* phenocopies^{37,38}. They do not look exactly like flies mutant for *bithorax* because only part of the haltere is transformed into wing. These changes can be produced only at or around the blastoderm period^{37,38}, suggesting that the phenocopying agent interferes with some determinative process that takes place at that stage³⁸.

This evidence from clonal analysis and phenocopies does not tell us when imaginal disks are determined; in neither case has the total extent of the marked or transformed tissue been defined and it could include internal or larval organs. As individual disks appear as identifiable structures at late embryonic stages^{39,40} they are probably determined by that time.

Determination and homoeotic genes

By the criteria usually used to define the state of determination, the homoeotic mutants change it. For example, antennal disks of *spineless-aristapedia* differentiate leg structures when transplanted into metamorphosing larvae⁴¹. In experiments where genetically marked cells from two different imaginal disks are dissociated, mixed together, and allowed to grow in an adult abdomen they usually sort out from one another^{26,27}. While wild-type antennal and leg disk cells will segregate from each other, antennal disk cells from flies mutant for *spineless-aristapedia* will mix with wild-type leg disk cells⁴¹. In similar experiments, mutant haltere disks for *bithorax* differentiate wing structures in isolation and their cells will mix with wild-type wing cells^{41,42}. Thus, by these criteria, the state of determination of the antennal disk is changed by *spineless-aristapedia* into that of the leg and the state of determination of the haltere is changed by *bithorax* into that of the wing. It follows that the normal function of homoeotic genes is necessary for the normal process of determination.

Since the disks become determined in early development, it is expected that the homoeotic genes will function early in development. There is some evidence that they do. In flies homozygous for extreme *bithorax* and *postbithorax* combinations (which transform the entire haltere into a complete wing, see cover), the number of cells of the mutant haltere disk is 2–4 times larger than the wild-type haltere disk in the first larval period and equal or similar to that of the wild-type wing disk⁴³. This result indicates that the mutant phenotype is already expressed by the first larval period and therefore that the wild-type gene has already been required.

There are several experiments which indicate that there is, as well, a late requirement for the *bithorax* gene. When *bithorax* clones are produced in a wild-type haltere, the clones show mutant phenotype^{3,43} (that is, they produce wing structures) even if they are induced before the end of the third larval period⁴³. Only when the clones are produced in the late third stage larva do they fail to show mutant phenotype⁴³. As we discuss below, the gene product may still be required after that time. A similar conclusion is indicated by some temperature sensitive alleles of *bithorax* where experiments have located the phenocritical phase in the third larval period⁴⁴. Clearly, the activity of the *bithorax* gene is required until late in development of the third instar, but not thereafter. This ability of wild-type phenotype to persist in the absence of wild-type genes has been described for certain mutants affecting bristles, and called *perdurance*⁴⁵. Although other explanations are possible, the simplest is that perdurance is the persistence in mutant cells of some wild-type function which is not diluted out in the last few cell divisions before the disk stops growing. This view is supported by the observation that late *bithorax* clones that show perdurance *in situ* (that is wild-type phenotype),

differentiate mutant phenotype after additional growth in culture *in vivo* (Garcia-Bellido, unpublished). We have seen that there is evidence for *bithorax* function in the first stage larva, and that it is needed until metamorphosis. It seems likely, therefore, that the gene is active throughout most of larval development, and perhaps in the embryo as well (see ref. 43 for discussion).

Although the preceding discussion relies heavily on data on *bithorax* mutants, observations on other homoeotic mutants point to similar conclusions: there is evidence that the *engrailed* gene is required throughout most of the larval period for normal development of the cells of the posterior wing^{6,46}. Its activity stops intermingling of anterior and posterior wing cells^{46,47}. Indeed, as the anterior and posterior cells are first segregated at the blastoderm stage^{34,35} but may lie adjacent to one another^{48,33} the *engrailed* gene is probably required then. Mitotic recombination^{6,47} and temperature shift experiments⁴⁷ show that wild-type function of the *engrailed* gene is required until the late third larval period. Mutant *spineless-aristapedia*, (*ss*⁴⁹), clones produced earlier than the mid-third larval period in a wild-type antenna show mutant phenotype (leg structures) but they retain wild-type phenotype when induced in the late third stage larva⁴⁹. Also, the cold-sensitive allele *ss*^{49a} has a phenocritical period in the third larval stage^{50,51}. Both these observations are most simply explained as perdurance effects. Thus the *bithorax*, *engrailed* and *spineless-aristapedia* genes probably have a similar mode of action; they are responsible for the expression of specific states of determination by groups of cells. Specific disk cells require a continuous supply of the wild-type product of these homoeotic genes for the maintenance of their state of determination. If the activity of the homoeotic genes fails, the state of determination changes accordingly.

It now becomes easier to understand the instability of the state of determination in some hypomorphic (leaky) mutations like *Antennapedia of Rappaport*¹⁰ or *Contrabithorax*⁸. In the first case the mutant antenna is a mixture of leg and antenna cells and in the second case the mutant wing is a mixture of wing and haltere cells. When clones homozygous for genetic markers are produced in the mutant appendages, it is found that even clones produced in the third instar can comprise leg and antenna in the case of *Antennapedia* and haltere and wing in the case of *Contrabithorax*. These experiments indicate that the decision whether to make one or another type of structure is not inherited through cell divisions but is made very late in development. Although these results have been interpreted as evidence for late determination in the mutant disks¹⁰ they probably instead reflect the inherently unstable state of determination of the mutant cells that can change according to the amount of wild-type product present in each cell. Even during *in vivo* culture the homoeotic genes and their products are still required: *spineless-aristapedia* 40a has a phenocritical phase in the middle of the third larval period^{50,51}. Mature antennal disks from the late third instar have passed the phenocritical period and have a strict state of determination which is independent of the temperature. However, if these same disks are cultured *in vivo*, the cells produced during the period of extra growth can become determined differently according to the temperature in which they grow⁵¹.

Compartments and homoeotic genes

Clonal analysis of imaginal disks of *Drosophila* and the abdomen of the milkweed bug, *Oncopeltus*, have shown that there are certain boundary lines in the adult cuticle which are not crossed by clones after a given moment in development^{52–55}. In *Drosophila* the introduction of the Minute technique^{4,56} allows the production of marked clones that grow more rapidly than surrounding cells and reach a very large size. These clones can nearly fill certain regions

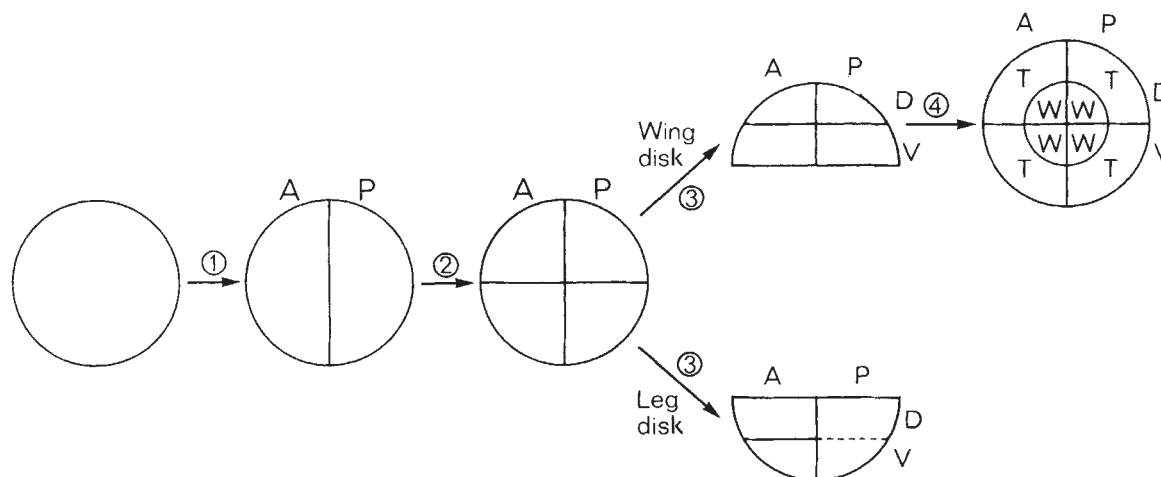


Fig. 2 Diagram of compartmentalisation of the mesothoracic cuticular structures. After each subdivision (represented by a straight line) no clone can include territories on both sides of that line. The first step (1) is the segregation of anterior (A) and posterior (P) polyclones, and this occurs, probably, at the blastoderm stage. The second (2) consists of the separation of the dorsal part of the segment (wing disk) and the ventral part (leg disk). This event takes place before 10 hours of development. These two disks later become physically separated. During the larval period the wing disk becomes subdivided (3) into wing dorsal (D) and wing ventral (V) and about the same time (4) into thorax (T) and wing blade (W). For the leg disk there is some evidence³⁴ of the subdivision of the anterior compartment into dorsal and ventral.

of the cuticle which are defined by precise boundaries in the adult. These regions are called compartments^{4,5}. They are constructed by all the descendants of a particular group of founding cells (a polyclone³⁷) which is set aside at an early stage of development. It has been shown that dividing cells cannot cross certain boundaries, and that some of these become effective before others^{4,5}, so that there is a successive subdivision of polyclones into daughter polyclones. In the cases studied so far the number of cells in the founding polyclones is small, being between 5–50 (refs 53, 54, 58–60, 61).

The assignment of a cell to a given polyclone is a stable developmental commitment; all the cell's progeny will be constrained to form structures within one specific compartment. In this way development can be visualised as a process in which homogeneous populations of cells are split into subpopulations with different developmental potential.

In *Drosophila* the formation of compartments has been studied for several imaginal disks^{4,5,34} and has been reviewed recently^{57,62}. Here we will discuss mainly the relationship between compartments, homoeotic genes and determination.

For the wing disk there are at least three subdivisions that produce eight major compartments. The wing is first subdivided into anterior and posterior regions and each subdivided further into dorsal, ventral, wing blade, and thoracic parts (Fig. 2). The first subdivision occurs so early that the mesothoracic disks (wing and second pair of legs) are subdivided into anterior and posterior polyclones before the wing-leg segregation occurs^{34,35}. This is shown by clones produced about the blastoderm stage of embryonic development which include both anterior wing and anterior second leg structures or posterior wing and posterior leg. No clone has been found to transgress the antero-posterior compartment boundary in either leg or wing^{34,35}. This observation shows that compartmentalisation begins before individual disks are formed. It is the whole mesothoracic polyclone (which forms the cuticle and perhaps other internal tissues as well) that becomes subdivided into anterior and posterior polyclones. The progeny cells of these anterior and posterior primordial polyclones will include all the anterior (leg and wing) and posterior (leg and wing) cuticular structures respectively. The probable sequence of compartmentalisation in the mesothorax is shown in Fig. 2.

An important feature of the antero-posterior compart-

ment boundary is that it apparently defines the limit of function of certain homoeotic genes^{4,5,46,47}. This is the case for the haltere-wing transformations produced by the *bithorax* and *postbithorax* mutants (both members of the *bithorax* pseudoallelic series³). The anterior wing structures produced by *bx³/bx⁺* (Fig. 1) in the haltere disk correspond to those defined in the ordinary wing by the antero-posterior compartment boundary, and the posterior wing structures produced by *pbx/pbx⁺* correspond to the posterior ones defined in the normal wing by the same boundary. Thus *bithorax* and *postbithorax* have an effect on specific polyclones so that entire compartments are transformed. The same boundary is also recognised by the mutation *engrailed*. The activity of the wild-type function of *engrailed* is required by posterior wing disk cells: without it, these cells fail to develop both a normal posterior compartment and a normal antero-posterior compartment boundary^{6,46,47}.

Of other compartment boundaries described for the wing disk, the one separating proximal (thoracic) from distal (wing) structures seems to be the limit of the transformation produced by *wingless*. This mutation produces a duplication of the thoracic structures while the wing disappears⁷. As it affects the haltere disk in a homologous manner, the *wingless* gene may be involved in the development of the distal compartments of both the wing and haltere disks⁸.

Determination begins when the original founder cells of the polyclone are chosen. Thereafter, during normal development, the cells of the polyclone can only form parts of a restricted region in the adult—the compartment. One would like to dissect the determinative process down to its operational elements, and some hints come from mutants like *engrailed* which produce duplicated compartments within one disk. It would seem that the subdivision of the polyclone into two sub-polyclones has proceeded normally, even though the homoeotic gene is defective. This suggests that, in normal development, there are at least two separable elements in each determinative step: (1) the definition of groups of cells and (2) the permanent activation of a homoeotic "selector gene"⁶² in one or more groups. Garcia-Bellido⁶² has coined the term selector gene to emphasise that there may well be more than one class of homoeotic mutation: homoeotic changes could result from mutations which affect the subdivision of polyclones, the genetic machinery leading to the

activation of a particular homoeotic selector gene, or the selector gene itself.

The choice of particular groups of cells is probably geographic⁵⁷ and depends, for example, on polarity and positional information in the egg^{63,64} or position in the disk. Homoeotic mutations in genes affecting these processes might be expected to have unusual properties, some might show a maternal effect. The gene products might only be required at an early stage of development, so that clones of cells mutant for these genes produced after that stage might show normal wild-type phenotype, *wingless* is, possibly, an example of such a gene⁸.

The selector gene, as exemplified by *bithorax*, is a gene whose product is required continuously throughout development to 'select' a particular developmental pathway⁶². The role of the selector genes is of considerable interest; they seem to control the activities of many other genes which perform executive functions⁶². For example, the cell marker mutants are not restricted to particular compartments and their products are probably required by all cells that secrete cuticle. When the selector gene product is defective or missing all compartments where that selector gene is normally activated will be homoeotically transformed—which explains why single genetic lesions such as *engrailed* can produce homologous transformations in different disks.

Conclusions

At least in some cases, homoeotic mutations transform entire compartments. This indicates that the activity of these genes is restricted to specific polyclones. As we have seen the activity of homoeotic genes is necessary for the expression of certain states of determination. Thus the compartments seem to be the units of determination during normal development, the founding polyclone of a given compartment constituting the initial group of determined cells.

In this view, the final state of determination of the cells belonging to a compartment is the result of a series of events. Every determinative event is accompanied by the activation of a certain selector gene. Some of these events are common to different polyclones, but the combination of them, and therefore the combination of active and inactive selector genes, is unique to each compartment. Determination is a result of genetically controlled commitments made by groups of cells and passed on to all their progeny. The effect of each determinative step is conditioned by the previous ones, so that at each moment the cells react according to their developmental history.

Are there compartments and homoeotic genes in vertebrates?

It is not clear whether compartments occur in vertebrate development, and if they do, whether they are similar to those found in insects. The idea of lineage segregation is of course inherent to the germ layer theory, and it has been suggested before that vertebrate organs of one cell type may have their origin in a special group of cells⁶⁵. Moreover, recent studies on early mouse development have shown there is a series of binary decisions, involving small numbers of cells (15-50, ref. 66), each apparently accompanied by changes in cell structure and possibly in cell adhesion, and each resulting in some restriction in developmental potential. For example, the first of these separates the prospective embryonic cells (inner cell mass) from the trophoblast. As in insects⁵⁷ these segregations appear to be made geographically⁶⁸.

In vertebrates determination has often been thought of as being to a particular cell type, such as muscle or nerve. Yet the picture coming from studies on *Drosophila* is one of commitment to a precise region; that is some kind of

area property⁵⁷. In vertebrates, detailed lineage studies could reveal whether a single organ or tissue is subdivided into compartments, but have not yet been undertaken. Other possible correlates of compartmentalisation might give further information. For example, some compartment boundaries in insects appear to be recognised by peripheral nerve axons⁶⁷, and a precisely placed line, which is respected by such axons, has been found separating the amphibian limb into two regions⁶⁸.

There is a lack of genetic evidence for compartments and homoeotic genes in mammals; this may not be significant since homoeotic mutants are more likely to be viable in insects, like *Drosophila*, where there is a complete metamorphosis and some adult structures (such as wings) are dispensable. These observations are certainly suggestive—even in the absence of detailed studies of cell lineage and the existence of frank homoeotic mutants.

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articles

Configuration of tobacco mosaic virus RNA during virus assembly

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When TMV reassembles, the uncoated RNA is folded back along the growing rod, probably down the central hole. This surprising configuration is essential for rapid elongation—presumably supplying RNA to its site of incorporation while keeping the bulk of the free RNA out of the way.

THE location of the nucleation region for the assembly of tobacco mosaic virus (TMV), from isolated coat protein and RNA, about 1,000 nucleotide residues from the nearer end of the RNA molecule (the 3'-hydroxyl terminus)^{1,2} raises questions about the elongation process. Since the RNA is totally enclosed in the mature virus particles, elongation from the original site of nucleation must occur in both directions—towards the 3'- and 5'-hydroxyl termini of the RNA. This presents a paradox in view of the observation³⁻⁷ that partially assembled rods of TMV have RNA tails only at one end, in agreement with the generally accepted view that assembly would be polar. We have shown that the two ends of the TMV rod can be distinguished in the electron microscope because one is convex and the other concave and, taking this together with stripping experiments, we have shown in which direction the RNA runs within the complete nucleoprotein helix⁸. We have now applied this technique to locate the free RNA in the growing nucleoprotein rods.

Preparation of rodlets

Partially assembled rodlets of the desired length range were obtained by allowing an appropriate amount of the protein, supplied as a disk preparation, containing about 80% of the 2-ring disk aggregate⁹, to reassemble with the RNA¹⁰ at 20 °C in sodium phosphate buffer, pH 7.0, ionic strength 0.1 for 10–15 min⁹. Rods

about half the size of complete virus particles were obtained with a protein:RNA ratio of 10:1. Rodlets with free RNA tails at the 5'-hydroxyl end were prepared by partial alkaline stripping as before⁸.

Both the rodlet preparations described were contaminated with free protein—either unreacted and possibly damaged in the partially assembled preparation or that which had been stripped off in the partially stripped preparation—and possibly some free RNA. These contaminants were much smaller than the rodlets and were removed before reassembly by centrifugation through sucrose gradients, without allowing the rodlets to pellet, as this alters their reassembly activities⁹. Gradients were prepared in Tris-chloride buffer, pH 8.0, ionic strength 0.1, by layering into tubes 2 ml 60% (w/v) sucrose, 2 ml 40%, 3 ml 20% and 2 ml 5% sucrose. Samples (3 ml) were layered on top and the gradients were centrifuged at 40,000 r.p.m. and 5 °C for 1 h in an MSE 6 × 14-ml titanium swingout rotor. Fractions were collected from the bottom of the tubes and the fractions from the turbid layer were pooled and dialysed against sodium phosphate, pH 7.0, ionic strength 0.1 containing 1 mM sodium azide.

The turbid suspensions had spectra characteristic of nucleoprotein, while the fractions from the tops of the gradients gave the spectrum of TMV protein, suggesting that little free RNA was present in the original preparations.

Location of RNA tails on growing rods

Although the uncoated RNA is not as clearly visible at the ends of partially assembled rodlets as on partially stripped rodlets, it can nevertheless be seen on many of the particles and the morphologically distinct ends of these particles can be distinguished. From Fig. 1 it seems that the RNA tails are at opposite ends of the two types of rodlet. This was confirmed by inspecting all the particles in

Fig. 1 Electron micrographs of TMV particles: *a*, partially stripped with alkali⁸, and *b*, partially reassembled. The images are aligned with the rounded or convex ends at the top and the concave ends at the bottom. The 'puffs' of uncoated RNA can be seen at opposite ends in the two cases (approximately $\times 170,000$). Specimens from the rodlet preparations were prepared by allowing the particles to adsorb on to carbon-coated grids from a drop of solution for about 30 s, followed by rinsing with distilled water and negative staining with 1% (w/v) uranyl acetate. The grids were examined and photographed in a Philips EM 300 electron microscope at a magnification of approximately 25,000.

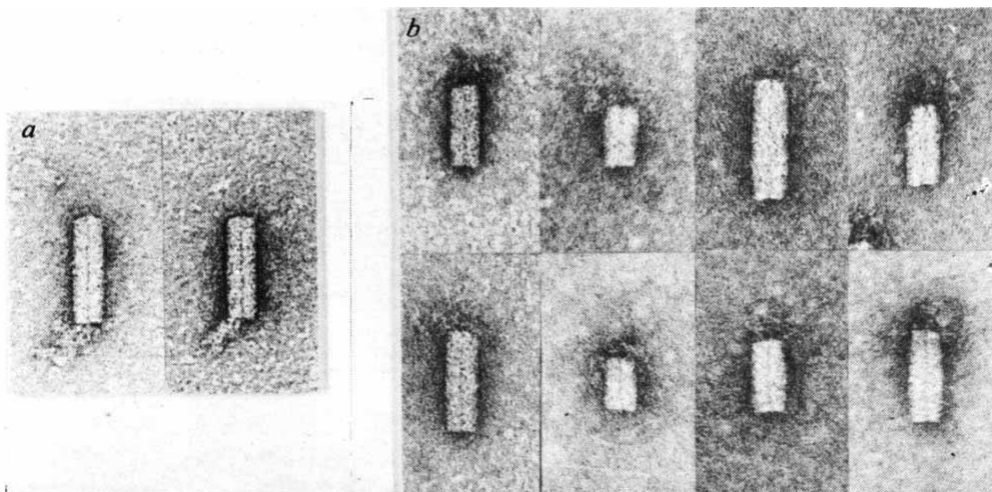


Table 1 Numbers of particles for which the end with the exposed RNA is morphologically distinguishable

Type of rodlet	End showing RNA		
	Convex	Concave	Both
Partially stripped ⁸	1	62	6
Partially assembled	88	4	3

several fields of partially assembled rodlets and assigning the free RNA to the different ends whenever this could be done unambiguously. The counts are shown in Table 1, together with those for rodlets partially stripped by alkali using the same technique⁸. Clearly the RNA tails of the partially assembled rodlets occur at the convex ends of the particles in contrast to the situation found for partially stripped rodlets⁸. (Less than 8% of rodlets, whether single or double tailed, were identified with a tail at the concave end.)

Because TMV RNA is about 6,400 nucleotides long with the nucleation region about 1,000 nucleotides from the 3'-hydroxyl end^{1,2} and hence more than 5,000 nucleotides from the 5' end, the main direction of assembly must be from 3' to 5'. In partially assembled rodlets the longer tail should therefore contain the 5' end, which is the end released by alkali stripping¹¹. As RNA tails are visible in partially assembled rodlets principally at the ends which contain the 3' ends of the RNA in the final viruses, the RNA must be folded back on itself in the growing rods because it protrudes only from the end at which least elongation occurs (Fig. 2).

Rates of elongation

We suggest that the looping back of the 5' end of the RNA in the growing rods, probably down the central hole, is important for the mechanism of elongation of the rods in the dominant direction, from 3' to 5'. In this case we expected that the rates of elongation of partially assembled rods and of partially stripped rodlets might be different because, although both would be elongating in the 3' to 5' direction, the long RNA tail would be tucked down inside the former but protruding from the stripped end of the latter.

This prediction was investigated using rodlets which had been purified and checked in the electron microscope to ensure that most still had RNA tails. Protein for elongation was supplied both as a disk preparation and as A-protein. The results of one such experiment, in which the partially assembled rodlets and two preparations of partially stripped rodlets were purified in parallel, including the final dialysis in the same flask, to eliminate any possible effect of preparative history, are shown in Table 2. Although the large molecular sizes and unknown molecular weights of the preparations make it unsafe to try to calculate the absolute rates of elongation from these rates of increase in turbidity, or even to make a close comparison of the rates between

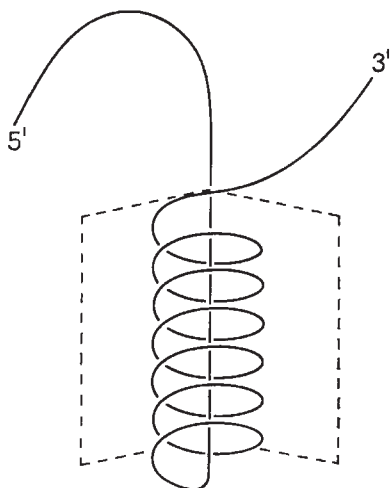


Fig. 2 Schematic picture of the suggested arrangement for the RNA in a growing rod. The dominant direction of assembly (that is, from 3' to 5') is downwards—at the end opposite the two RNA tails. The placing of the longer, 5' tail up the central hole is arbitrary and, while we think this is the most likely configuration, there is no direct proof as to how it reaches the opposite end of the rod.

different preparations, the partially assembled rodlets seemed to elongate more than an order of magnitude faster than did the partially stripped rodlets. This conclusion remains, even allowing for the greater concentration of the former.

A compatible result was obtained when the lengths of partially stripped rodlets were compared by electron microscopy before and after incubation with a disk preparation at 1.0 mg ml⁻¹ for 30 min. While partially assembled rodlets would, in these conditions, be expected to elongate by 150–200 nm (refs 9,12), the partially stripped rodlets only increased by about 10 nm. Although a ² analysis on the histograms of lengths of the rodlets showed that even without genuine elongation, such an apparent change could have arisen from sampling with a probability of just under 0.1, we think that elongation had taken place, as it has a 90-probability of doing. Moreover, rods partially stripped from the same end with SDS⁸ will reassemble over several hours to give nuclease resistant infectivity¹³, in agreement with the idea that some slow elongation can probably occur even with the RNA tail free at the abnormal end of the growing rod.

Preliminary location of both tails

The observations of the RNA tails described above were made with negatively stained specimens, so that high resolution electron microscopy distinguished the ends of the rodlets, but in these conditions the RNA tails only appear as 'puffs' and it is not

Table 2 Rates of reassembly of partial TMV rodlets

Rodlet preparation	Rate of turbidity increase (D ₃₁₀ per min)	
	Disk preparation	A-protein
Partially stripped I	0.00125	0.0006
Partially stripped II	0.00115	0.00065
Partially assembled	0.0316	0.0221

Rodlets were prepared either by partial alkali stripping or by partial reassembly. Elongation in sodium phosphate, pH 7.0, ionic strength 0.1 at 20° C, was followed by the increase in turbidity at 310 nm (ref. 9), with either a disk preparation or A-protein added to 1 mg ml⁻¹ final concentration. Final rodlet concentrations were 0.20 and 0.195 D₂₆₀ per ml for the partially stripped rodlets I and II and 0.42 D₂₆₀ ml⁻¹ for the partially assembled rodlets (corresponding to approximately 0.6, 0.5 and 1.2 mg ml⁻¹, respectively).

possible to distinguish between one or two tails at the same end of a rod, but rather the major tail (the 5' end) dominates the image. We have occasionally seen partially assembled particles apparently with two RNA tails coming from the same end, by electron microscopy of specimens shadowed after spraying onto carbon coated grids with only bovine serum albumin as a spreading agent. We do not regard these observations as very significant because most of the particles showed only a single tail, and some free RNA was visible in the background. To obtain better spreading and separation of the tails it is probably necessary to add some denaturing agent. Preliminary experiments, however (unpublished results of B. Jacrot and D.Z.), showed that partially assembled rods are very unstable when RNA is denatured for spreading, although partially stripped rodlets could be visualised. This again shows the difference between these types of rodlet, but emphasises the need for extreme caution in interpreting such data because preparation may significantly alter the specimen.

An alternative approach to visualise and distinguish the ends of the RNA is to label them with a bulky molecule. We have done some preliminary experiments using ferritin, activated for reaction with periodate-oxidised RNA¹⁴ by coupling to carbonylhydrazide with ethyl dimethylaminopropyl-carbodi-imide^{15,16}, to label both termini. One problem is that the tendency to form ferritin aggregates could cause confusion. Although we have never seen a particle with ferritin molecules at both ends, we have seen several partially assembled rodlets on which two ferritin molecules appear, in the electron microscope, to be attached to the same RNA 'puff' and yet are too far apart to be attached directly to each

other. We believe that this indicates that the two RNA termini are indeed at the same end of the assembling rodlet and we are continuing experiments to confirm this.

Implications for assembly

The structure of the protein disk is known to a resolution of 5 Å (ref 17) and consists of two rings, each of 17 protein subunits, stacked on top of each other. While these make a firm contact at high radius, they are separated vertically at the RNA-binding radius, like a pair of 'jaws', and the protein has considerable flexibility inside this radius. These features suggested that the RNA might well be intercalated between the two rings of the disk, during both nucleation and elongation, from the central hole of the disk and in this way the two-layer disk could be incorporated into the single helix.

Although there is no firm evidence of whether the doubling back of the longer RNA tail occurs inside or outside the growing nucleoprotein helix, we suggest that it is probably inside with the RNA strand running freely down the central hole (Fig. 2). This configuration in the growing rod would follow smoothly from a mechanism of nucleation by the insertion of a hairpin loop of RNA

into the centre of the first disk¹⁸. Such a geometry will allow access of the relevant region of the RNA to its binding sites on both rings of an incoming protein disk and yet hold the bulk of this RNA strand well out of the way of the region of activity, greatly facilitating the process of rod elongation.

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A model for superlight velocities of extragalactic radio sources

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An explanation is proposed for apparent superlight velocities of compact radio sources. In our model high velocities are a consequence of the interaction of the radio sources with the ambient gas in a galactic nucleus or quasar. This model predicts that the pair of sources is not centred on the nucleus and that the individual components should be smaller and more separated when observed at higher frequency.

APPARENT faster-than-light velocities of separation have been measured with VLBI for compact double radio sources in 3C120, 3C273, 3C279, and, most recently, in 3C345 (refs. 1-8). Furthermore, in a number of low-frequency sources the time-scale for luminosity changes corresponds to apparent expansion velocities in excess of the speed of light⁹.

Many explanations have been offered for these perplexing observational results; however, the models proposed so far either contain implausible assumptions or predict phenomena which have not been observed, such as double sources in which the components move toward each other or in which the components have very dissimilar luminosities^{3-7,10-13}.

From observations of extended extragalactic radio objects it is known that mechanisms exist for ejecting energy sources in well defined directions from the centres of galaxies and quasars^{11,15}. We show here how the motion of one of these energy sources through the ambient gas in the centre of a quasar or galactic nucleus can produce a pair of bright radio emission regions which separate at superlight velocities or can produce rapid luminosity changes. The model we propose thus provides suggestive connections among the observed properties of quasars and galactic nuclei, and extended and compact radio

sources. Our model is an extension of the expanding radio source models of Shklovskii^{16,17} and van der Laan¹⁸ which have been remarkably successful in describing the variable emission from 3C120 (ref. 19). In their models a stationary source creates a spherical cloud of relativistic electrons and magnetic field which expands adiabatically. While the source is highly compact, it is optically thick due to synchrotron self-absorption. As the cloud expands, the flux at a given frequency increases until the cloud reaches some critical radius, at which it becomes optically thin at this frequency; thereafter the flux decreases.

Our model differs from earlier models in that we allow the source of particles and/or field to move through an ambient medium of varying density. It is easy to see that apparent superlight velocities can be obtained by this generalisation. Consider the evolutionary sequence in which a source moves out from the centre of a galaxy or quasar with a velocity $v_s < c$. As the energy source moves through the ambient gas it creates a cavity in its wake which is filled with particles and magnetic field. In the regions where the cavity has expanded to a critical radius R_c , corresponding to unit optical depth, the radio surface brightness is at a maximum. This bright region—the apparent position of the radio object—lags behind the actual energy source by a distance $v_s \tau_{exp}$ where τ_{exp} is the time for the cavity to expand to R_c . If the cavity is confined by the ram pressure of an external medium of density ρ , then the expansion velocity is proportional to $\rho^{-1/2}$ and $\tau_{exp} \propto \rho^{1/2}$ (ref. 20). Because the density of the ambient gas decreases away from the centre of the galaxy or quasar, the lag distance decreases as the source moves outward. The apparent position of the radio-emitting region tends to catch up to the energy source. The apparent velocity is thus greater than the actual physical velocity of the energy source. Because this apparent velocity is merely due to a phase-delay effect—the delay of the brightening of the radio object is greater when it is embedded in higher density gas—arbitrarily large velocities could, in principle, be obtained.

A single moving energy source can actually produce two bright emission regions which separate at superlight velocities. If

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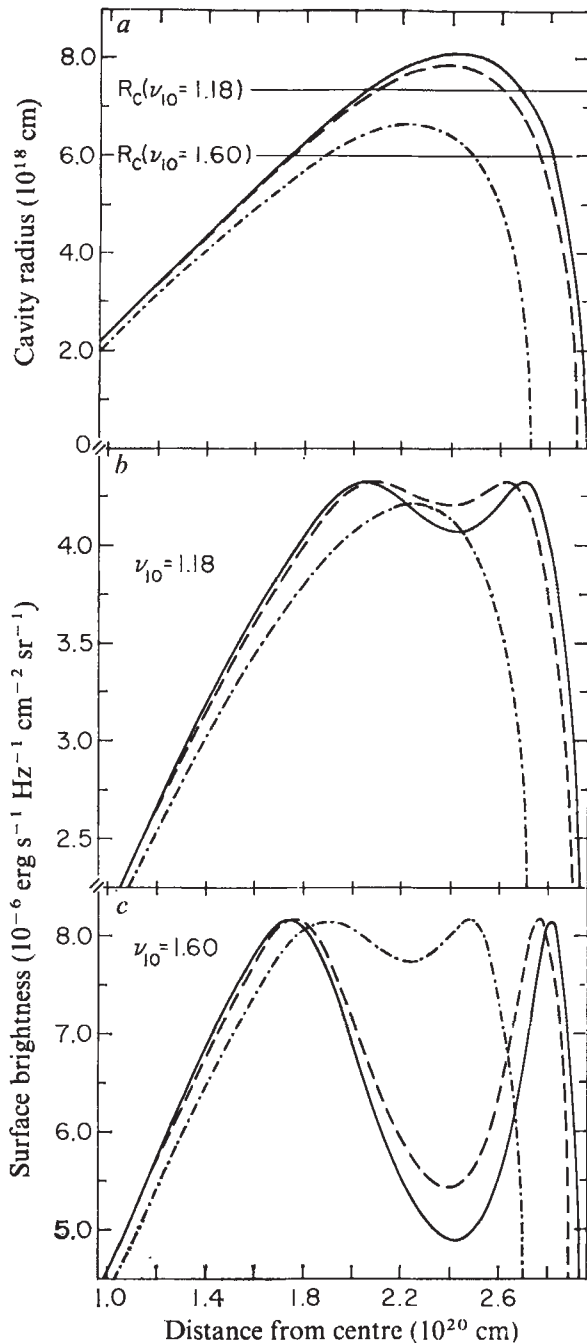


Fig. 1 The evolution of a compact radio object is illustrated at three epochs. *a* The evolution of the cavity boundary. The critical radii for two frequencies are indicated here. *b*, *c* The evolution of the surface brightness along the symmetry axis of the cavity for each of these frequencies. ---, $t = 1.830 \times 10^{10}$ s; - · -, $t = 1.955 \times 10^{10}$ s; —, $t = 1.980 \times 10^{10}$ s.

the ambient gas is sufficiently concentrated toward the centre of the galaxy or quasar, the cavity expands very slowly in these dense regions and has its maximum diameter somewhere between the centre of the quasar or galaxy and the location of the energy source. This point is illustrated in Fig. 1. Figure 1*a* shows an example of an evolutionary sequence for the cavity radius; the critical radii for two frequencies are indicated. Figure 1*b* and *c* show the corresponding evolution of the brightness distribution.

Consider the cavity to be divided into disk-like segments of fixed thickness along the direction of motion. As each segment expands, the brightness at a given frequency increases until the segment reaches a critical radius; the brightness then decreases as the segment expands further. As long as the radius of the cavity is everywhere less than the critical radius, the brightness

distribution exhibits a single maximum which coincides with the position of the maximum cavity radius (cf., brightness distribution for $\nu_{10} = 1.18$ at $t = 1.83 \times 10^{10}$; where ν_{10} is the frequency, units 10^{10} Hz). With further expansion, some portions of the cavity exceed the critical radius. The brightness distribution then becomes bimodal and the regions of greatest brightness occur where the cavity radius is equal to the critical radius.

When the maximum radius of the cavity is only slightly greater than the critical radius, the two brightness maxima separate with arbitrarily large velocity, but the brightness distribution has a very shallow minimum. During the subsequent evolution the apparent separation velocity decreases and the bimodal structure of the brightness distribution becomes more pronounced.

As the energy source moves into regions of lower density, the separation between the leading bright region and the source decreases due to the decreasing τ_{exp} . The leading bright region therefore moves forward faster than the energy source. The trailing object moves towards the centre of the galaxy or quasar with a smaller, but comparable velocity so that the two bright regions separate with an apparent velocity which is significantly greater than the velocity of the energy source and which can easily be superluminal.

This mechanism for producing rapidly separating pairs of bright regions has a well-defined signature which may be deduced from the frequency dependence of the critical radius and the geometrical nature of the model. Since synchrotron-self absorption decreases with frequency^{21,22}, the critical radius is larger at lower frequencies as indicated in Fig. 1*a*. As can be seen from the figure, a pair of bright regions observed simultaneously at two different frequencies should have the following characteristics: at the lower frequency which corresponds to a larger R_c (1) the two sources are closer together, (2) the brightness minimum between the two sources is less well defined and (3) the individual component radii which are roughly proportional to R_c are larger.

To make more quantitative estimates of the physical structure and brightness distribution of a radio source, one must formulate an explicit and hence more elaborate model. Any such model requires a number of simplifying assumptions about the gas distribution in the centres of galaxies and quasars, about the nature of the energy source and about the mechanisms which operate within the cavity. A model of this sort, though at best heuristic, can serve to illustrate the general interrelationships among the properties of compact objects, galaxies and quasars and extended radio sources.

The model

The gas density in the galaxy or quasar is assumed to be constant near the centre and to decrease at some power law at large distances, z , from the centre according to the relation

$$\rho(z) = \rho_0(1 - z/z_0)^{-n} \quad (1)$$

where ρ_0 is the central density and z_0 is a core radius. The energy source moves away from the centre of the density distribution with a constant velocity v_s and deposits energy at a uniform rate in the form of relativistic particles and field.

It is assumed that each segment of the cavity evolves independently in two dimensions; that is, energy flows along the length of the cavity are assumed to be small compared with the work done by the cavity in expanding in the radial direction. This approximation is reasonable for the model presented here. For the highly elongated cavities, the pressure gradients along the cavity are small in the regions of interest; thus the time scale for energy flow along the cavity should be long compared with the typical radial expansion time.

The rate of expansion of the radius of the cavity at a given distance, z , is determined by the balance of the internal pressure, P_i , and the ram pressure of the external medium, ρv^2

$$\dot{r}(z, t) = (P_i/\rho(z))^{1/2} \text{ for } t > z/v_s \quad (2)$$

The internal pressure derives from the magnetic field, assumed to be chaotically oriented, and from the relativistic electrons and heavy particles. The energy densities of the magnetic field and of the relativistic electrons are, respectively, $U_M = B^2/8\pi$ and $U_e = \delta U_M$. The internal pressure is defined as κU_M , where the parameters δ and κ are constants of the energy source. For each slice of the cavity, the evolution of the magnetic field is prescribed by the relations

$$B = B_0 \quad \text{for } 0 < r \leq R_0 \quad (3a)$$

and

$$B = B_0 (r/R_0)^{-1/3} \quad r > R_0 \quad (3b)$$

The first phase (equation (3a)) is the input of energy at a rate

$$W = 3\kappa B_0^2 R_0^2 v_s/4 \quad (4)$$

This rate corresponds to filling the cavity of radius R_0 with particles and magnetic field and to accelerating the ambient gas of the cavity to the velocity given by equation (2). During the second phase (equation (3b)), the cavity expands adiabatically in two dimensions.

To complete the description of the model the energy distribution of the relativistic electrons must be specified. Initially, the distribution is taken to be a power law in energy at high energies

$$N(E) dE \propto E^{-m} dE \quad E > E_c \quad (5)$$

where $m > 2$. A specific choice of m , E_c and δ defines the proportionality constant in equation (5). We assume that the evolution of the spectrum is entirely determined by adiabatic changes which preserve the power law spectrum and require that $E \propto B^{1/2}$. This implies that other energy loss processes are either inconsequential or are counterbalanced by ongoing electron acceleration. In the case where the source velocity is perpendicular to the line of sight, the intensity of the radiation segment of the cavity of length dz is approximated by

$$I dz = 2r dz \frac{\eta}{\mu} (1 - \exp(-\frac{1}{2}\pi r \mu)) \quad (6)$$

where η and μ are the vacuum synchrotron emissivity and opacity, respectively. Expressions for η and μ are taken from refs 21 and 22. The surface brightness is approximately $I/2r$.

The parameters chosen for the model illustrated in Fig. 1 are $v_s = 1.5 \times 10^{10} \text{ cm s}^{-1}$, $\rho_0 = 1.72 \times 10^{-9} \text{ g cm}^{-3}$, $z_0 = 2.2 \times 10^{19} \text{ cm}$, $n = 10$, $B_0 = 3.8 \text{ G}$, $R_0 = 1.4 \times 10^{18} \text{ cm}$, $\delta = 0.01$, $\kappa = 2$, $m = 3$. The parameters are chosen so that the expansion velocity is non-relativistic during the period of interest. The two frequencies $\nu_{10} = 1.18$ and $\nu_{10} = 1.6$ correspond to observing wavelengths of 3.8 and 2.8 cm for a source with a redshift $z = 0.5$.

At the first epoch illustrated in Fig. 1, the source is about 600 yr old, but the double structure, the large apparent separation velocity and high luminosity are just beginning to emerge. The second and third epochs are separated by 8.3 yr, characteristic of an observing interval. Between the second and third epochs, the peaks of the brightness distribution at both frequencies separate with average velocities greater than c . The low frequency brightness peaks are 40% closer together and separate at a velocity about 25% higher than the high frequency brightness peaks.

For the parameters of our model B_c , the field in the region of the cavity where the radius equals R_c , is $\sim 0.1 \text{ G}$.

An electron which radiates at about 10^{10} Hz in this field has a radiative lifetime of the order of a few years, which is much shorter than the age of the source. As presently formulated, the model thus requires that some mechanism continually

replenish the higher energy electrons. This requirement is not a serious problem for the model. The necessity of some sort of *in situ* acceleration is a feature of many radio source models⁵, and the model parameters can be chosen to make the energy in the magnetic field sufficient to compensate for radiation losses for several thousand years.

For $B_c \sim 0.1 \text{ G}$, the rate of energy input to the cavity (equation (4)) can be estimated as

$$W \sim 2 \times 10^{46} \kappa (R_c/R_0)^{2/3} (v_s/c) \text{ erg s}^{-1} \quad (7)$$

(see equations (3b) and (4)). The total energy which is supplied to a cavity of length Z is WZ/v_s . For a cavity 100 pc long, this energy is of order 10^{57} erg .

The ambient gas density at the point, z_c , where the double source structure first appears can be derived from equation (2). Integration of this equation from time z/v_s to t yields an expression for the cavity radius. Solving for the gas density at the location of the maximum cavity radius gives

$$\rho(z_c) \sim 10^{-22} \kappa \left(\frac{B_c H_p c}{R_c v_s} \right)^2 \text{ g cm}^{-3}. \quad (8)$$

Here $H_p \equiv \rho/(d\rho/dz)$ is the density scale height at z_c . There is approximately a factor of 2 uncertainty in equation (8) due to the choice of R_0/R_c .

In order to achieve large apparent velocities the density scale height has to be of the same order as the separation of the bright regions; H_p/R_c is less than about 10. For this sort of density gradient equation (8) yields densities of 10^{-21} to $10^{-18} \text{ g cm}^{-3}$. The central density of the model shown in Fig. 1 is actually much greater than the above estimates; however, this high density is an artefact of the simple density distribution which was used (equation (1)) and does not provide an important physical constraint on the model.

The skewing of the cavity apparent in Fig. 1a is directly related to the steep density gradient. The lower gas density near the energy source allows a more rapid radial expansion causing distension towards the source. The positions of the leading bright regions at different frequencies, as determined by the intersections of the cavity boundary with the critical radii in Fig. 1a, are thus fairly close together. For steeper density gradients, the positions of these bright regions would be even less frequency dependent.

Discussion

We have shown that a source of relativistic electrons and magnetic field which moves out through the gas in the centre of a galaxy or quasar can produce two bright emission regions which separate at superlight velocities. The energy source must travel at a few tenths the speed of light and must deposit about 10^{57} erg over several tens of pc. Our model is not dependent on the specific nature of the energy source or the detailed mechanism for providing directionality. The source is perhaps a rapidly moving condensed object^{23,24} or simply a region where a beam of plasma interacts with the ambient gas²⁵⁻²⁷. The densities and scale heights which are required to yield rapidly separating double radio sources are consistent with the estimates of the properties of galactic nuclei and quasars which are deduced from optical studies²⁸.

The basic phenomenon described here has several distinctive characteristics which are essentially model independent: the bright regions should be smaller, more separated and have deeper minima between them when observed at higher frequencies. Furthermore, the pair of bright regions should not be symmetric with respect to the centre of the optical brightness distribution of the galaxy or quasar.

Within the context of the specific model formulated here, the structure of the bright regions is sensitive to the gas density scale-height: the structure is less frequency dependent when the density gradient is steeper. In active galactic nuclei or quasars

the required steep density gradients would develop as a result of violent events. Some of the assumptions which were made to develop the simple model shown in Fig. 1 should be relaxed to correspond more closely to the actual physical reality. One might, for example, expect to find irregularities in the density distribution, in the rate at which the source supplies energy, or in the source velocity. These may lead to fluctuations in the brightness of the components and in variations in the apparent velocity of separation. In addition, the source velocity need not be perpendicular to the line of sight. Interesting relativistic effects occur when a bright region moves at a high or superluminal velocity at small angles with respect to the line of sight^{11,12,29}. The apparent separation velocities of the bright components can be enhanced, and a single bright region moving toward the observer may appear as two rapidly separating components.

The expansion velocity of the source itself may be relativistic. Existing models which employ relativistic expansion to explain rapid variability in radio sources would imply extremely high velocities in some exceptional cases⁹. This rapid variability is more easily explained in terms of a highly extended cavity which expands relativistically: because the cavity can be elongated by a factor of 20 or more when maximum brightness is attained, the surface over which coherent expansion occurs is greatly increased. It should be noted that rapid variations observed at one frequency may indicate that at higher frequencies the source may appear as two rapidly separating components.

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letters to nature

A simple physical model for X-ray burst sources

THE observational properties of X-ray burst sources have recently been reviewed by Grindlay¹ and Lewin². These sources are concentrated in the direction of the galactic bulge and near the galactic plane. If they are at a mean distance of ~ 10 kpc and the burst radiation is emitted isotropically, then the time-averaged luminosities in bursts are typically $\sim 10^{34}$ – 10^{36} erg s⁻¹. Several of these sources may also have relatively steady X-ray luminosities³. Because of the limited sky coverage of available X-ray astronomy satellites, it seems likely that there are many times more burst sources in the galaxy than have heretofore been discovered.

There are ~ 150 known "steady" galactic X-ray sources³, which are also concentrated toward the galactic bulge and in the galactic plane and have luminosities of up to $\sim 2 \times 10^{38}$ erg s⁻¹. The most widely accepted model for many of these sources consists of a compact object that is accreting matter from a close binary stellar companion⁴. For many of the galactic-plane sources with identified optical counterparts, the companion stars are OB supergiants, and there is some evidence that these stars nearly fill their critical potential lobes (see, for example, refs 5 and 6). The wind emitted by the companion star may well be enhanced by its proximity to its critical lobe^{6,7}. Although the evolutionary time scale of an OB star from the main sequence to the supergiant stage is about

10^6 – 10^7 yr, it has been argued on statistical and theoretical grounds that a binary system containing such a star and a compact object becomes a bright X-ray source for only $\sim 10^4$ – 10^5 yr preceding the filling of the critical lobe of the OB star (ref. 7; ref. 8 and references therein).

The above information suggests a simple physical model for an X-ray burst source in the galactic disk, comprised of an unevolved OB star with a relatively weak stellar wind and a compact object in a close binary system. For some (as yet undetermined) reason, the stellar wind from the OB star is unable to accrete steadily on to the compact object unless the accretion rate, and hence the average X-ray luminosity, exceeds some critical value. Thus, when the stellar wind is sufficiently weak, the compact object accretes irregularly, leading to X-ray bursts. If this picture is correct, then many present burst sources will evolve into bright "steady" X-ray sources when the associated OB stars evolve and approach their critical lobes.

Accretion by a compact object from the weak stellar wind of a companion is also a tenable model for the burst sources associated with the galactic bulge. However, the stellar population of the galactic bulge is probably very different from the disk population, and the evolutionary histories and other detailed properties of the bulge burst sources are thus likely to be different from those of the disk burst sources. (The apparent association of three burst sources with globular clusters (refs 1 and 2 and references therein) strongly supports the identifica-

tion of a subset of these sources with Population II objects.)

A magnetised neutron star is the most plausible candidate for the compact object in an X-ray burst system⁹. Theoretical studies have indicated that the character of spherical accretion on to a magnetised non-rotating neutron star changes¹⁰, and that steady accretion might in fact not occur (F. K. Lamb, A. C. Fabian, J. E. Pringle, and D. Q. Lamb, submitted to *Astrophys. J.*), if the time-averaged X-ray luminosity $\lesssim 10^{36}$ erg s⁻¹. The rotation period of the neutron star may also play a critical role in determining the steadiness of the flow⁹.

For rotating neutron stars with sufficiently low luminosities or sufficiently short rotation periods, the corotation velocity in the vicinity of the Alfvén surface will be larger than the circular Keplerian orbital velocity. This results in an effective centrifugal barrier^{9,11} that should inhibit the steady accretion of matter and provide a mechanism for the storage of matter above the neutron-star surface (ref. 9; ref. 12 and references therein). For a $\sim 1 M_{\odot}$ neutron star of radius $\sim 10^6$ cm, corotation at the Alfvén surface is determined by the following approximate relation (see refs 11, 13) between the rotation period, P , the time-averaged luminosity, L , and the neutron-star magnetic moment, μ

$$P \sim 0.2 \left(\frac{L}{10^{37} \text{ erg s}^{-1}} \right)^{-3/7} \left(\frac{\mu}{10^{30} \text{ G cm}^3} \right)^{6/7} \text{ s} \quad (1)$$

For more rapid rotation, matter is likely to accumulate and compress the Alfvén surface until accretion on to the neutron-star surface can proceed. Under these evidently unstable conditions, bursting behaviour may result.

The observed values of X-ray luminosity and rotation period for the nine known binary X-ray pulsars are shown in Fig. 1. It is likely that these objects are all rotating magnetised neutron stars undergoing accretion of mass from binary stellar companions (ref. 14 and references therein). The relation $P \propto L^{-3/7}$

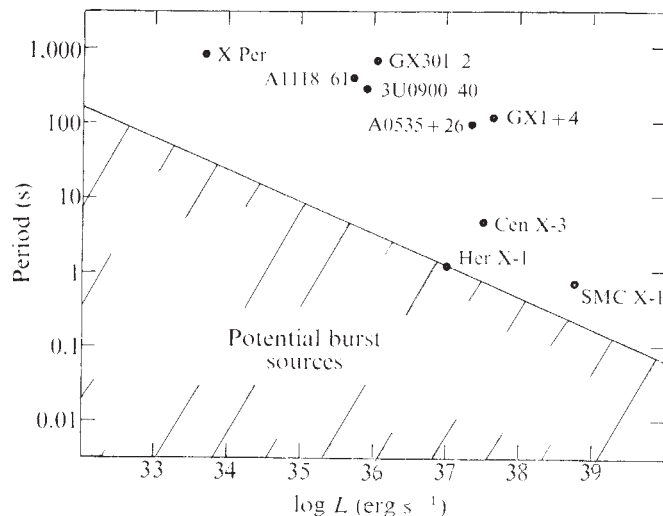


Fig. 1 Observed values of typical X-ray luminosity, L , and pulse period, P , for nine X-ray pulsars (see ref. 19). (For the transient sources A1118-61 and A0535+26, the maximum observed luminosity is indicated. The indicated pulse periods and luminosities are taken from ref. 20 and references cited therein. The straight line with logarithmic slope of $-3/7$ represents the relation between X-ray luminosity and pulse period such that matter in a circular Keplerian orbit will corotate with the neutron star at the Alfvén surface^{11,13,19}. The line is normalised so as to pass through the parameter values of Hercules X-1 (see text). For neutron stars which lie below this line, matter that reaches the Alfvén surface cannot corotate because its rotational velocity would then exceed the circular Keplerian orbital velocity, and such matter may therefore be unable to accrete steadily across the surface^{9,11}.

is superimposed on the figure and normalised so as to pass through the parameter values of Hercules X-1, which seems to be in a near balance between spinup and spindown (ref. 12 and references therein). Most of the X-ray pulsars lie substantially above this line; the 'missing' neutron stars that lie below this line may be X-ray burst sources.

We note that X-ray bursts have been observed from the direction of the bright 'steady' sources Serpens X-1 (3U1837+04), A1905+00, MX1728-33, and 3U1820-30, to within positional accuracies of $\sim 0.1^\circ$ (ref. 2 and references therein). Other burst sources may also have substantial 'steady' luminosities (refs 1, 2, and references therein). If the observational identification of bursts with bright 'steady' sources is confirmed, then the presence of bursting behaviour in a binary stellar system containing a compact object must depend upon additional parameters besides the time-averaged luminosity. For example, if the burst sources are rotating neutron stars, then the neutron-star rotation period and magnetic moment presumably also have a role in determining the existence of bursting behaviour⁹ (see equation (1)).

If our suggested identification of burst sources with binary systems containing compact objects is correct, then several predictions are possible. First, as more burst sources are discovered, the spatial distribution of the burst sources should follow the distribution of the 'steady' galactic sources (although the ratio of galactic-bulge objects to disk objects may be different for the burst sources than for the 'steady' sources). Second, the ratio of the total number of disk burst sources to "steady" disk sources should be comparable to the ratio of the main-sequence lifetime of an OB star to its lifetime in a state of enhanced stellar wind as it approaches its critical potential lobe (that is, $\sim 10^1$ - 10^3). Third, for some sources with slowly varying 'steady' X-ray luminosities, one should see transitions between bursting behaviour when the time-averaged luminosity is low and no bursts when the luminosity is high. This phenomenon has already been observed in the source 3U1820-30 (ref. 15). Other examples of this type of effect might be the onset of bursting behaviour during the extended inactive states of transient sources and Hercules X-1-type systems. Fourth, the existence of very large bursts from relatively nearby binary systems ($\lesssim 1$ kpc) is predicted. For some of these sources, it may be relatively easy to identify the optical companion star. It has previously been suggested^{16,17} that the high-energy tail of nearby X-ray bursts could account for the observed γ -ray burst events¹⁸. However, we note that the repetitive nature of the bursts from many of the X-ray burst sources is apparently not characteristic of the γ -ray sources.

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Detection of change in rotation measure of the Vela pulsar

OBSERVATIONS made over the past six years show that the rotation measure of the Vela pulsar has increased by 14%, while over the same period the dispersion measure has shown a marginally significant decrease of about 0.2%. These results imply that a region containing a relatively strong magnetic field has moved across the path to the pulsar. This region of strong field is most probably associated with filamentary structure in the Vela supernova remnant.

Using a polarimeter giving simultaneous measurements of all four Stokes parameters¹ we obtained observations of PSR 0833–45 at well separated frequencies on three occasions, December 1971, July 1975 and June 1976, as shown in Table 1. Except for the 338 MHz measurements, data were obtained at several frequencies within the bands given in Table 1, the measurements being simultaneous at either two or three frequencies within a band. Observations in different bands were separated by a few days for the 1971 and 1976 observations and by 3 weeks for the 1975 observations. These observations permitted accurate determinations of the rotation measure along the path to the source and of the position angle of the emitted radiation extrapolated to infinite frequency, the intrinsic position angle. The results of these measurements are given in the table and are plotted in Fig. 1 together with data from Komesaroff *et al.*^{2,3}, who observed the source in May 1970 at a number of frequencies simultaneously using a single linearly polarised feed at each frequency. The rotation measure of the Vela pulsar was determined at earlier epochs by Radhakrishnan and Cooke⁴ and Ekers *et al.*⁵, but their data are less precise than the more recent measurements and so are not included in Table 1. All rotation measures have been corrected for the effects of Faraday rotation in the Earth's ionosphere using concurrent measurements of the critical reflection frequency f_oF_2 . The correction was in the range of 0.7–1.3 rad m⁻² for these observations and is estimated to be uncertain by less than 10%.

It is clear from Fig. 1 that there has been a significant change in the rotation measure along the path to the source during the past 6 yr. The fact that there has been no significant change in the intrinsic position angle confirms that the observed changes result from variations in the rotation measure rather than in the emitted position angles. It also provides an *a posteriori* justification for the assumption of a ν^{-2} position angle dependence between the different frequency bands.

For the 1975 and 1976 observations, dispersion measures were also computed from the 631–649 MHz data; the results

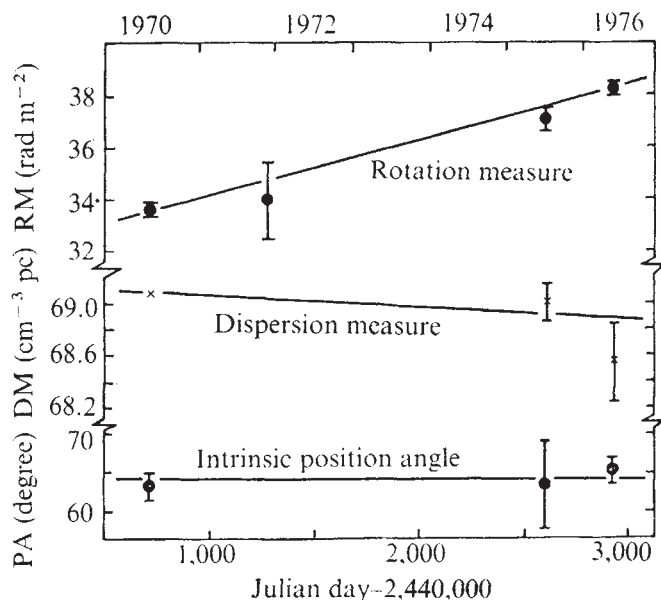


Fig. 1 Rotation measure, dispersion measure and intrinsic position angle of PSR 0833–45 at different epochs. For the rotation and dispersion measures the lines represent least-squares fits to the data, whereas for the intrinsic position angle the line has zero slope.

are shown in Table 1 and Fig. 1. There is some indication that the dispersion measure has decreased between 1970 and 1976.

For the rotation and dispersion measure data, the straight lines in Fig. 1 represent weighted least-squares fits to the observations and have slopes of 0.77 ± 0.04 rad m⁻² yr⁻¹ and 0.033 ± 0.030 cm⁻³ pc yr⁻¹ respectively (1 σ errors). If the dispersion measure change is real and associated with the rotation measure change, then the implication is that a region of excess electron density has moved out of the path to the pulsar during the past 6 yr. Since the rotation measure increased during this time, the mean line-of-sight component of the magnetic field in this region must have been directed toward the pulsar giving a negative rotation measure contribution. This is in the opposite sense to the mean interstellar field in this direction.

The short timescale of the changes suggests that the region concerned must be relatively small. One possibility is that the changes occurred within the pulsar magnetosphere itself. There is, however, good evidence that little or no Faraday rotation occurs within this region⁶. We therefore assume that the changes are taking place within the interstellar medium. The scale of the region may be estimated from the relative velocity of the pulsar and the medium. It is likely that the highest relevant velocity is that of the pulsar; observations of pulsar proper motions^{7,8} show that pulsars typically have velocities in excess of 100 km s⁻¹. The offset between the position of PSR 0833–45 and the centre of symmetry of the Vela supernova remnant together with the pulsar characteristic

Table 1 Rotation measure, dispersion measure and intrinsic position angle for PSR 0833–45

Date	Observing frequencies (MHz)	Rotation measure (rad m ⁻²)	Dispersion measure (cm ⁻³ pc)	Intrinsic position angle (°)	Reference
May 1970	300, 410, 467, 628, 1410	33.6 ± 0.1	69.08 ± 0.01	63.1 ± 1.5	2, 3
December 1971	338, 395–400	33.9 ± 1.5	—	—	Present work
July 1975	631–649, 1612–1720	37.0 ± 0.17	68.99 ± 0.13	63.5 ± 5.7	Present work
June 1976	268–278, 631–648	38.17 ± 0.06	68.53 ± 0.3	65.2 ± 1.5	Present work

age suggests that the Vela pulsar may have a transverse velocity as large as 500 km s^{-1} . If we adopt this figure as a reasonable upper limit to the velocity, an interval of 6 yr corresponds to a transverse scale length of $3 \times 10^{-3} \text{ pc}$ or 600 AU for the electron density and field fluctuation. If we further assume that the line-of-sight dimension of the region is comparable, then the excess electron density corresponding to a dispersion measure change of $\sim 0.20 \text{ cm}^{-3} \text{ pc}$ is about 65 cm^{-3} . Of course, if the region has smaller dimensions the electron density would be greater.

Since this electron density is high relative to normal interstellar values we can approximate the weighted mean line-of-sight component of the magnetic field in the time-varying region by

$$B_l = 1.23 \Delta(\text{RM})/\Delta(\text{DM})$$

where $\Delta(\text{RM})$ and $\Delta(\text{DM})$ are in their conventional units and $\langle B_l \rangle$ is in microgauss. For $\Delta(\text{RM}) \sim 4.5 \text{ rad m}^{-2}$ and $\Delta(\text{DM}) \sim 0.2 \text{ cm}^{-3} \text{ pc}$ we have $\langle B_l \rangle \sim 30 \mu\text{G}$. This estimate will be too high if the electron density in the region concerned is larger than that assumed, either because of an underestimate of the dispersion measure change or because of the presence of a non-varying component. The estimate will be low if the dispersion measure change is less than that assumed.

It is well known that supernova remnants contain filaments of enhanced optical and non-thermal radio emission. The small indicated size of the time-varying region, together with the relatively strong magnetic field and high electron density values derived above, suggests that the observed changes result from the motion of a filament in front of the pulsar. The derived parameters are well within the range of those found for filaments in supernova remnants. For example, Duin and van der Laan⁹ deduce that filaments in IC 443 have typical dimensions of 10^{-3} to 10^{-2} pc , magnetic field strengths of $150 \mu\text{G}$ and electron densities of 250 cm^{-3} . Bell *et al.*¹⁰ deduce even larger field strengths for regions of intense radio emission in Cassiopeia A, about $3,000 \mu\text{G}$. An H α survey of the Vela supernova remnant by Elliott *et al.*¹¹ showed no evidence for an intense filament in the vicinity of the pulsar. However, diffuse nebulosity was detected in this region. The present results suggest that this nebulosity contains small-scale structure and regions of enhanced magnetic field.

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Observational consequences of synchrotron self-Compton models of compact extragalactic X-ray sources

RECENT measurements¹⁻⁶ have confirmed the existence of a class of extragalactic X-ray sources quite different from those associated with clusters of galaxies. These sources (Cen A, NGC4151, NGC1275, 3C390.3 and 3C273) are characterised by flat X-ray spectra, low-energy X-ray absorption, strong optical emission-line spectra and a compact radio or millimetre component⁴. These observations give strong impetus to the construction of models^{7,8} in which these phenomena are related. We report here some of the results of a synchrotron self-Compton model⁹ (SSC) which we feel can account for many of these observational properties. This model includes the effects of adiabatic expansion and synchrotron losses. We concentrate here on two of the three most studied sources—NGC4151 and NGC1275—and report our results on Cen A elsewhere. We use the results of this analysis to make tentative observational predictions about the colours of the nuclei of potential X-ray sources.

In this SSC model the non-thermal radio, X-ray, and optical emission originate in an extremely small region located in the nucleus of the galaxy. The non-thermal flux causes photoionisation of the gas in this nucleus which results in the observed strong emission-line optical spectrum. In addition this gas absorbs low-energy X-rays causing a turnover in the X-ray spectrum.

We can determine several physical properties of this emitting region, assuming X-ray emission due to SSC radiation, that is, that X rays are produced by the Compton scattering of lower energy synchrotron photons off the radiating population of relativistic electrons. Two relations between observables are⁷:

$$B \approx 7.42 \times 10^{-60} i_{a0}^2 v_n^5 \theta_s^4 F_{v_n}^{-2} \text{ gauss} \quad (1)$$

and

$$F_{v_x} \approx F_{v_n}^2 (v_x/v_n)^{-\alpha} v_n^\alpha \theta_s^{-2} B^{-(1-\alpha)} \quad (2)$$

$$[2.75 \times 10^4 e_{a0} (2.79 \times 10^6)^{-(1-\alpha)} \ln \Lambda] \text{ erg cm}^{-2} \text{ s Hz}$$

where F_{v_x} is the X-ray flux at the frequency v_x , θ_s is the angular size of the source (radians), B is the magnetic field in the emitting region, F_{v_n} is the radio or millimetre flux at the synchrotron self-absorption frequency v_n , and i_{a0} , e_{a0} and $\ln \Lambda$ are functions of order unity discussed by Jones *et al.*⁷. We have assumed isotropic disordered magnetic fields and no re-isotropisation of pitch angles.

Assuming that the hard $E^{-0.4}$ energy X-ray spectrum of NGC 4151^{1,10}, which exists out to at least 100 keV, originates by the SSC process in a component for which v_n is in the millimetre band, as is required by recent upper limits (M. Werner 1976, H. Hudson and T. Soifer 1975, personal communications), we determine that $\theta \approx 1.4 \times 10^{-5} \text{ arc s}$ (~ 1.5 light d at 19 Mpc (ref. 11)), $B \approx 2 \text{ G}$, and that the synchrotron lifetimes of electrons responsible for the 2–10 keV X rays is $\approx 7 \text{ d}$. Here we neglect any effects due to adiabatic expansion. The model predicts that a sudden injection of a large number of relativistic particles would result in an X-ray flare for which the rise and decay times would each be a few days. The time scale of flux increase is that of the filling of the emission region by relativistic particles; the time scale of decay is that of synchrotron energy losses. We expect the X-ray spectrum to steepen with both time and energy during the decay period, so that the length of an observable flare should be shorter at higher X-ray energies. The X-ray spectral index should be

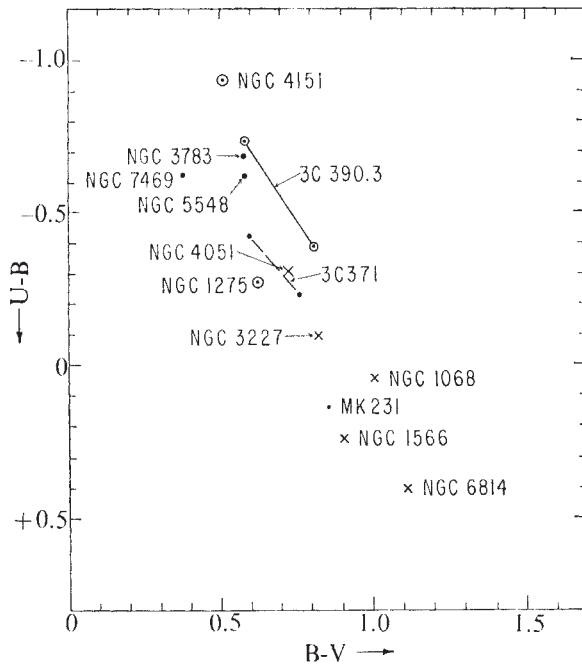


Fig. 1 The colour-colour plot for the nuclei of selected active galaxies. The lines represent the various colours seen in the nuclei of 3C390.3 and 3C371. The crosses mark those sources shown not to have detectable X-ray emission, the circles those known at this time to emit X-rays, and the dots are objects unobserved in the X-ray. The relative contribution of non-thermal radiation to the optical flux of a galactic nucleus increases from lower right to upper left in the U-B, B-V plane.

about the same as the radio (millimetre) index up to a high frequency radio break. Above this break frequency, the source should produce a power law infrared (IR) and optical flux with index $\alpha_{IR} \approx (4\alpha_{X-ray} + 3)/3$, where $\alpha_{X-ray} = \alpha_{mm}$ is the X-ray energy index. Since $\alpha_{X-ray} \approx 0.39$ (Ives *et al.*²) we expect $\alpha_{IR} \approx 1.52$, which is close to our $\alpha_{IR} \approx 1.46$ power law fit to the 2.2–21 μ m data of Rieke and Low¹².

This model predicts a detectable flux of ~ 10 Jy at 300 μ m from NGC 4151, that there should be no correlation between the MHz radio and X-ray flux, and that the X-ray absorption should be due to material in the forbidden line and broad-emission-line regions of the optical nucleus (ref. 2 and G. Shields and R.M., unpublished).

A similar model can be developed for NGC 1275 using the recent radio data of Pauliny-Toth *et al.*¹³. Here observations give $\nu_n \sim 8.5$ GHz, $F_{\nu_n} \sim 71$ Jy and $\alpha_{radio} \sim 0.45$ if we assume a single, extremely compact component. To explain the measured X-ray flux from the core of the Perseus cluster¹⁹, presumed to be due to the nucleus of NGC 1275, we derive $\theta_s \sim 1.4 \times 10^{-3}$ s (2.1 light years at 100 Mpc), $B \sim 0.1$ gauss, synchrotron lifetimes of ≈ 10 y and $\alpha_{X-ray} \approx 0.45$. The measured VLB¹³ angular size is $\approx 1.3 \times 10^{-3}$ s and Burbidge *et al.*¹⁴ find a typical time for variability in the compact radio component of 12 yr. We expect a non-thermal IR and optical flux with spectral index $\alpha_{IR} \sim 1.6$ which agrees well with a power law fit to the aperture-corrected data of Penston *et al.*¹⁵, for which $\alpha \approx 1.56$. It is also expected that NGC1275 should show absorption in its X-ray spectrum and that there will be a correlation between X-ray emission and the intensity and turnover frequency of the GHz radio emission.

In this SSC model the non-thermal optical and infrared flux in the nuclei of these objects is related to the X-ray flux. Thus there should exist a correlation between the relative strength of the non-thermal component in the nucleus of a galaxy and the probability of X-ray emission from it. Sandage¹⁶ has shown a

relationship between the position of a nucleus in the U-B, B-V plane and the strength of the non-thermal component. In Fig. 1 we show the colour-colour diagram for a sample of well studied low redshift active galaxies. Following Sandage, the colours of the nuclei^{16–18} of these galaxies have not been corrected for reddening. We note that all those known as of June 1976 to have X-ray emission (NGC4151, NGC1275, 3C390.3) lie in the part of the diagram corresponding to strong non-thermal components while those objects known not to emit X rays¹⁹ (NGC1068, NGC1566, NGC3227, NGC4051 and NGC6814) lie in a region corresponding to weak non-thermal components. Based on this correlation we suggest that a search be made for X-ray emission from NGC3783, NGC5548, NGC7469 and 3C371.

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Absolute scale of thermoelectricity

I HAVE obtained the absolute thermoelectric power of lead between 10 and 350 K by direct measurement of the Thomson heat for the first time. Above 20 K the values are higher than the present scale by up to $0.30 \mu\text{V K}^{-1}$. Acceptance of the new values would require the recalculation of the thermopowers of almost all metals, since lead was almost certainly the reference material for the thermocouple measurements which were used to determine them. The size of the correction makes it particularly significant for the theoretical interpretation of the thermopowers of many metals and alloys.

The presently accepted scale of the thermopower of lead is that of Christian, Jan, Pearson and Templeton¹. They calculated the thermopower S from the voltage V of a lead against superconductor thermocouple and from the Thomson heat μ of lead of Borelius and coworkers (ref. 2 and many earlier papers), as

$$S(T) = \int_0^{T_1} \frac{d^2 V}{dT^2} dT + \int_{T_1}^T \frac{\mu}{T} dT \quad (1)$$

where T_1 was about 17 K. In fact the Borelius values for μ were obtained indirectly from measurements of the Thomson heat of an alloy and the thermopower of lead relative to the same alloy. The Borelius measurements were made more than 40 years ago and have never been tested below 100 K by comparison with direct measurements of the Thomson heat.

In the present work two identical lead wires were placed in a small temperature gradient ΔT and a direct current i was passed through them (going up the gradient in one wire, down it in the

other). The change in temperature δT at the midpoint of one wire on reversal of the current direction was measured by means of a differential thermocouple. (By symmetry, δT is half the change of the temperature of the midpoint of one wire with respect to the temperature of the midpoint of the other wire.) From the general solution given by Nettleton³ for the temperature distribution in the wire, it can be shown that the Thomson heat can be calculated from

$$\mu = 4k A \delta T / iL \Delta T \quad (2)$$

where k is the thermal conductivity; A , the cross-sectional area; and L , the length of each wire. A full description of the experiment will be published later.

The present values of μ/T agree within 5% with the superconducting experiment¹ from 10 to 17 K. The values are several times larger than the earlier values from 20 to 40 K, reconverge at 80 K, and stay within 10% of the earlier values to 290 K.

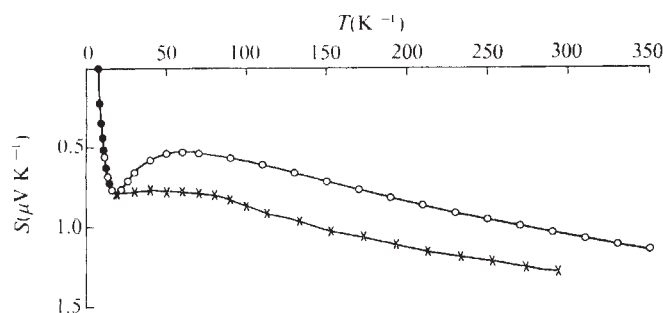


Fig. 1 Absolute thermopower of lead. ○, Present result; ●, Christian *et al.*¹ thermocouple experiment; ×, Christian *et al.*¹ compilation of Borelius² measurements.

The small region of mismatch of μ/T has a large effect on the thermopower scale. In Fig. 1 the present results have been tied to the old scale at 17 K. The agreement in the overlapping region from 10 to 17 K is within $0.02 \mu\text{V K}^{-1}$. Above 17 K the two scales diverge rapidly to be $0.24 \mu\text{V K}^{-1}$ apart at 50 K. This difference is approximately maintained at higher temperatures. The new curve is much smoother than the old, and it resembles the thermopower curves of other pure metals, having a well defined phonon drag peak. The thermopower of lead and the difference ΔS between present work and that of Christian *et al.*¹ are given in Table 1. Linear interpolation into ΔS can be used to transform thermopowers on the old scale to the new scale within $0.01 \mu\text{V K}^{-1}$.

The measurements on lead were confirmed by the following experiments. The thermopower of a silver–1.2% gold alloy was calculated from 15 to 290 K from measurements on a lead-

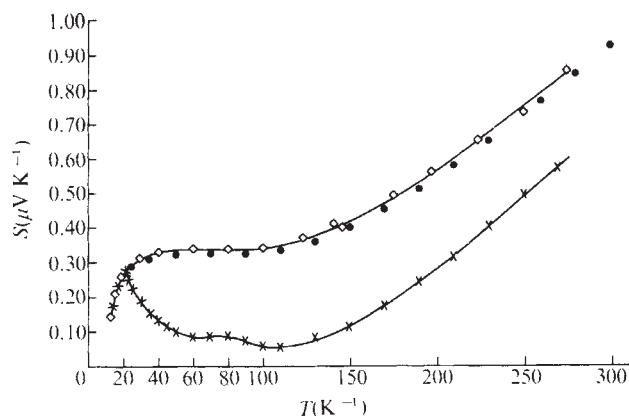


Fig. 2 Absolute thermopower of silver–1.2% gold alloy. ●, From direct measurement of Thomson heat; ◇, thermocouple experiment: Roberts scale for S_{pH} ; ×, thermocouple experiment: Christian *et al.* for S_{pH} .

alloy thermocouple using the new curve for lead. The Thomson heat of the alloy was also measured directly from 15 to 290 K and its thermopower calculated by integrating equation (1) upwards from the thermocouple result at 17 K. The thermopowers agreed within $0.02 \mu\text{V K}^{-1}$ over the whole range (see Fig. 2). This test is particularly sensitive to the effect of systematic errors in the Thomson heat measurements because, at most temperatures, the Thomson heat of the alloy is of opposite sign to that of the lead. Any significant heat loss from the sample (the most probable source of error) will cause the magnitude of the observed μ to be too small in each case. The errors are therefore additive. Only if both samples lose no heat will the thermopowers calculated from the Thomson heat results agree with those observed in the thermocouple experiment. These experiments confirm that the new scale is correct within $0.02 \mu\text{V K}^{-1}$.

Figure 2 also serves as an example of how correcting thermopowers by means of Table 1 can be expected to produce drastic changes in the thermopowers of some alloys. On the Christian *et al.* scale the phonon drag peak for the silver–gold alloy is very narrow. On the new scale it appears very broad indeed, possibly decaying as T^{-1} to room temperature.

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Fission/ α analysis of the U, Th families

A MODIFICATION is presented here of the technique of particle track analysis (PTA) by means of which it is possible to measure in particular geologic materials not only uranium but also thorium and/or radioactive disequilibria in the U and Th families.

PTA based on slow-neutron-induced fission is by now a standard method for U analysis (ref. 1, and references therein), but the technique has not been so successful for Th. Methods have been proposed based on the fission of Th by various energetic particles^{2–5}, but few actual analytic studies have been carried out on geologic materials, probably because of the difficulties associated with providing fluxes of such energetic particles which are both sufficiently large and known with sufficient accuracy.

The method proposed here is based both on the slow-neutron fission of U and on the natural α -decay of U, Th, and their radioactive daughters. Cellulose nitrate (CLN) records α -tracks, as other plastics such as Lexan record fission tracks⁶. If a flat surface of a U, Th-bearing material is placed against a sheet of

Table 1 Preliminary values of the thermopower of lead and correction to scale of Christian *et al.*¹.

T (K)	S ($\mu\text{V K}^{-1}$)	ΔS ($\mu\text{V K}^{-1}$)
17	0.78	0.00
20	–0.78	0.00
25	–0.72	0.06
30	–0.66	0.12
40	–0.58	0.19
50	–0.54	0.24
60	–0.53	0.25
80	–0.54	0.25
100	–0.58	0.28
150	–0.71	0.30
200	–0.83	0.29
250	–0.95	0.26
300	–1.05	—
350	–1.14	—

$\Delta S = S_{\text{Roberts}} - S_{\text{Christian}}$. A more closely spaced table will be published later.

Table 1 Th analyses of known geological materials; U determined by fission track analysis, Th according to equation (1)

Sample	U (p.p.m.)	R_u	R_{obs}	R_{th}	Th (p.p.m.)	Th (other work)
1. Spergen (limestone)	1.1	0.00249	0.00253	4×10^{-5}	0.06 ± 0.06	$0.1 \pm 0.1^\dagger$
2. NBS-611* (Glass)	462	0.174	0.346	0.172	410 ± 50	$455 \pm 3.6^\ddagger$
3. NBS-613* (Glass)	37.5	0.014	0.026	0.012	29 ± 7	$37.55 \pm .09^\ddagger$
4. Tektite 1	$2.1 \pm 0.6^{**}$	0.00479	0.0134	0.00861	12.1 ± 2.2	$13.0 \pm 2.3^{**}$
Tektite 2 (Indochinites)	$2.1 \pm 0.6^{**}$	0.00479	0.0131	0.00831	11.7 ± 2.2	$13.0 \pm 2.3^{**}$
5. Enkaf 1 (Mn-rich deposit)	4.1	0.0102	0.0106	0.0004	0.5 ± 0.5	$0.68 \pm .01^{\dagger\dagger}$

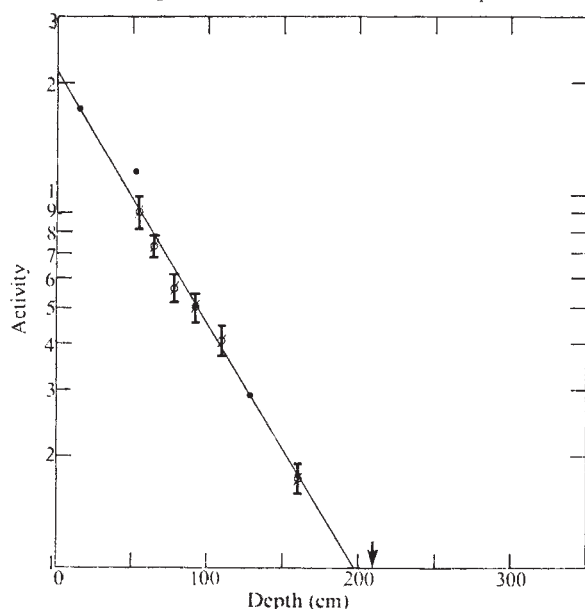
*Corrected for disequilibrium due to isotopic fractionation and $T < T'$ † H. Rydell, personal communication, 1972 (α -spectrometry) ‡ Ref. 7 ** Ref. 10 (Indochinite mean) †† Ref. 11

CLN, naturally-occurring α -particles from the U and Th families will impinge on the CLN and leave detectable tracks. The total α -activity of the sample is thus measured. The sample (a disk of pressed powder) is then placed in contact with a sheet of Lexan and irradiated by slow neutrons, for normal fission track analysis of the U alone.

The density of α -particle tracks in the CLN is given by $\rho = Rt$, where R is the rate at which α -tracks are produced per minute per cm^2 and t is the time the sample is in contact with the CLN. R is given by

$$R = W^{1/2}(k_u U + k_{th} Th) \quad (1)$$

with W the atomic weight calculated on the atomic fraction basis; k_u and k_{th} constants depending on the decay constants, α -ranges, and counting criteria; and U and Th indicating concentrations in p.p.m. This expression based on the empirical Bragg-Kleeman relation, was first published by Cherry⁷, who used it to describe the total α -particle activity measured by thick-sample scintillation counting techniques. I have determined the k -values for the present work by measuring the density of tracks produced in CLN exposed to materials of widely-differing W : U metal foil, ThO_2 powder, and artificial glasses with known contents of U and Th^{5,8}. These values will vary depending on the individual investigator's criteria for identifying tracks in the CLN; my values are $k_u = 5.15 \times 10^{-4}$, $k_{th} = 1.6 \times 10^{-4}$.

Fig. 1 α -Activity in core V19-61. ●, Excess ^{230}Th activity in d.p.m. g^{-1} (ref. 9). ○, R values, normalised at 93 cm depth.

With the U content of the sample given by the fission track analysis, the Th content can be calculated from equation (1). Table 1 shows the results of some determinations on a variety of geological materials of known U and Th contents.

The time t necessary for the accumulation of α -tracks is a function of the U, Th contents and the accuracy desired. Densities of 100 tracks cm^{-2} are easily measured, but one will normally desire at least several hundred tracks for statistical considerations. Samples with $U = 1$ p.p.m. and $Th/U = 4$ will generate densities of 500 tracks cm^{-2} in about two months. The uncertainty in the determination of Th in these conditions is about 8%.

This method of Th analysis is valid only for those samples in which the U and Th families are in radioactive equilibrium, since it is the total α -activity of the sample that is measured. This is an important but not too severe a geologic restriction. Perhaps even more important are certain types of sample which do not meet the conditions of radioactive equilibrium. Those samples which have lost or gained any of the radioactive daughters gradually or non-quantitatively, by such processes as weathering or migration, must simply be discarded, but there are certain geological processes which establish radioactive disequilibria in precise conditions, and these may be investigated by this technique.

One particular example is the deposition of deep-sea sediments, in which an excess of ^{230}Th over that established by radioactive decay from ^{238}U is deposited, owing to the much lesser solubility of Th than U in water. As the sediment is buried by succeeding debris, this ^{230}Th (being unsupported by any longer-lived parent) decays away with its characteristic half life of 7.5×10^4 yr until equilibrium is established at a lower activity level by the U present. The decay of excess ^{230}Th is one means of establishing deep-sea sedimentation rates⁹. Other nuclides in the U and Th families may also be deposited out of equilibrium, but previous studies have shown that ^{230}Th is much the dominant species.

Measurements of the total α -activity in such sediments, corrected for α -activity in equilibrium with the U present, should show this characteristic decline with depth unless significant postdepositional mobility of members of the U, Th families should separate the various components of the total α -activity and thus disturb the correlation. Figure 1 shows the results of such an investigation into core V19-61 collected by the Lamont Observatory from near the East Pacific Rise, for which an excess ^{230}Th chronology has been established⁹. The straight line is the best fit through the ^{230}Th points: clearly the total α -activity data show the same slope, and therefore the same sedimentation rate as that obtained by direct measurement of the ^{230}Th .

The advantage of the fission/ α -particle (f/α) technique is its simplicity and rapidity. Although experimental data on deep-sea sedimentation rates are of extreme value, there are very few such data in the literature because of the laborious procedures involved in the wet chemistry and α -spectrometry necessary for ^{230}Th measurements. On the other hand, all the f/α data of Fig. 1 represent one day's work. General applicability of the f/α method to this problem will depend on the question of postdepositional

mobility of the U, Th families in deep-sea sediments; the lack of such mobility observed in core V19-61 may not be a common feature of ocean basins. The ease of the method will greatly facilitate the necessary surveying studies.

Many similar geological situations exist, in which the f/α method may be useful. Sediments with very high rates of sedimentation may be analysed by measuring the growth of the ^{226}Ra daughter of the excess ^{230}Th (half life 1622 yr) for the first few thousand years and then the decay of the ^{230}Th and its daughters after the Th-Ra equilibrium has been established. Corals incorporate U preferentially to Th, and so the growth towards equilibrium of ^{230}Th rather than its unsupported decay may be used as a chronometer in this case. In general, the method should be applicable to any geological situation in which a precise disequilibrium is established at a particular time, and in which the total α -decay is dominated, aside from U, by one nuclide and its daughters.

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Light-driven, carrier-mediated electron transfer across artificial membranes

WE report that light-induced electron transport across artificial membranes mediated by electron carriers has been realised by photogeneration of the reducing species.

Quinone-type carriers achieve redox processes across membranes²⁻⁴ and are involved in mitochondrial and photosynthetic electron and proton transport (ubiquinones, plastoquinones, vitamin K)⁴⁻⁶. They perform cotransport (symport) of two electrons and two protons through their reduced hydroquinone form. Ferrocene type carriers⁴ operate by way of oxidation to the ferricinium cation.

Electron transfer experiments have been conducted in a cell in which an aqueous solution of a reducing agent (RED: sodium dithionite or ascorbic acid, reduction potentials $E_0 = -1.13$ and $+39$ V respectively)⁷ is separated from an oxidising aqueous solution (OX: potassium ferricyanide, $E_0 = +0.36$ V)⁷ by a diphenyl ether membrane supported on a Millipore filter and containing a suitable carrier molecule. The carriers used were either menadione (vitamin K₃: 2-methyl-1,4-naphthoquinone, NQ, $E_0 = +0.44$ V)⁷ or dibutyl ferrocene (DBF, $E_0 = +0.23$ V)⁸. The electron transfer process is followed by monitoring the reduction of ferricyanide to ferrocyanide at 420 nm, where only the former absorbs. In the absence of carrier in the membrane no reduction is observed. When either NQ or DBF are dissolved in the membrane, ferrocyanide is formed in the OX phase showing that electron transfer occurs through the NQ/NQH₂ and DBF⁺/DBF redox carrier systems (Table 1). The transport rate increases with increasing concentrations of NQ in the membrane although not linearly (Table 1, runs 3-6). The absence of leakage through the membrane

was checked at the end of the experiments by ensuring that no iron hexacyanide had passed into the RED compartment.

Coupling electron transport to light irradiation should be possible in a system where irradiation would generate a reducing species which would then be able to transfer an electron to a carrier molecule through the interphase, but which would not itself cross the membrane. Methyl viologen (MV^{2+} , 1,1'-dimethyl-4,4'-bipyridinium chloride) seemed to be a suitable substance; it has been used in many physico-chemical and biological (for example, in photosynthesis) redox studies. Reduction of the colourless oxidising form (MV^{2+}) gives a blue cation ($\text{MV}^{+\cdot}$) ($E_0 = -0.44$ V)⁷, which is able to reduce quinones like vitamin K₁² and being mono-charged should remain in the water phase. Furthermore, MV^{2+} may be photoreduced in aqueous solution by a photosensitised reaction involving visible light, a sensitising dye, proflavine (PF, $E_0 = -0.73$ V; $\lambda_{\text{max}} = 445$ nm), and an electron donor, ethylenediamine tetraacetic acid (EDTA)⁹.

We have used this system (PF + MV^{2+} + EDTA) as the RED phase in transport experiments with menadione as the electron carrier in the membrane and ferricyanide in the OX solution, as in previous experiments. When the transport cell is kept in the dark no change in colour or in ferricyanide concentration was observed. When irradiated with visible light from a halogen lamp the RED phase turns rapidly blue, showing that $\text{MV}^{+\cdot}$ is produced, and the ferricyanide concentration decreases. If the light is turned off, the ferricyanide concentration levels off; on reillumination it continues to drop. No change in ferricyanide concentration is observed in the absence of menadione in the membrane. These results show that light-coupled electron transport has indeed been achieved by the photoactivated process shown in Fig. 1a. Table 1 (runs 10-13) and Fig. 1b show some typical experiments.

Table 1 Electron transport by carrier molecules across an artificial membrane in chemical (runs 1-9) and light-coupled (runs 10-15) redox systems

Run	RED (mM)	Carrier in membrane (mg)	Transport rate ($\mu\text{M} \times \text{h}^{-1}$)
1	Asc. 5	NQ(6.8)	1.3
2	Asc. 5	—	0
3	Asc. 50	NQ(1.5)	0.4
4	Asc. 50	NQ(3.2)	1.8
5	Asc. 50	NQ(5.8)	3.1
6	Asc. 50	NQ(10.9)	5.0
7	Dit. 10	NQ(7.2)	24.7
8	Dit. 10	—	0
9	Asc. 10	DBF(7.2)	3.0
	PF (μM)	MV ²⁺ (mM)	EDTA (mM)
10*	40	1.0	20.5
11*	0	1.0	20.7
12*	0	0	20.0
13*	0	0	0
14*	54	1.0	20.5
15*	0	0	20.5

All experiments were performed at 25 °C under argon in a cell consisting of three phases: RED, reducing aqueous solution (40 ml) membrane OX, oxidising solution (40 ml); the two aqueous solutions are stirred continuously. Membrane: 75 ± 3 mg carrier solution in diphenylether on a Millipore filter (cellulose nitrate 4 cm diameter, 500 Å holes). Carriers: NQ, 2-methyl-1,4-naphthoquinone menadione; DBF, dibutylferrocene. Runs 1, 2: OX, 5 mM potassium ferricyanide, 0.2 M potassium phosphate buffer at pH 7.05 in both RED and OX. Runs 3-8 and 10-15 OX, 10 mM potassium ferricyanide, 0.1 M potassium phosphate buffer at pH 7.0 in OX and at pH 6.2 (runs 3-6) or pH 7.0 in RED. Run 9: OX, 20 mM potassium ferricyanide, 0.1 M sodium citrate buffer at pH 6.37 and 1.2 mM sodium bromide in both RED and OX. Asc., ascorbic acid; Dit, sodium dithionite; PF, proflavine; MV^{2+} , methyl viologen dichloride; EDTA, ethylenediamine tetraacetic acid. In the photoreduction experiments (runs 10-15) the concentration of each component of the RED phase is indicated.

*Light source: runs 10-13: 150 W halogen lamp; runs 14 and 15: 200 mW argon laser at 476.5 nm.

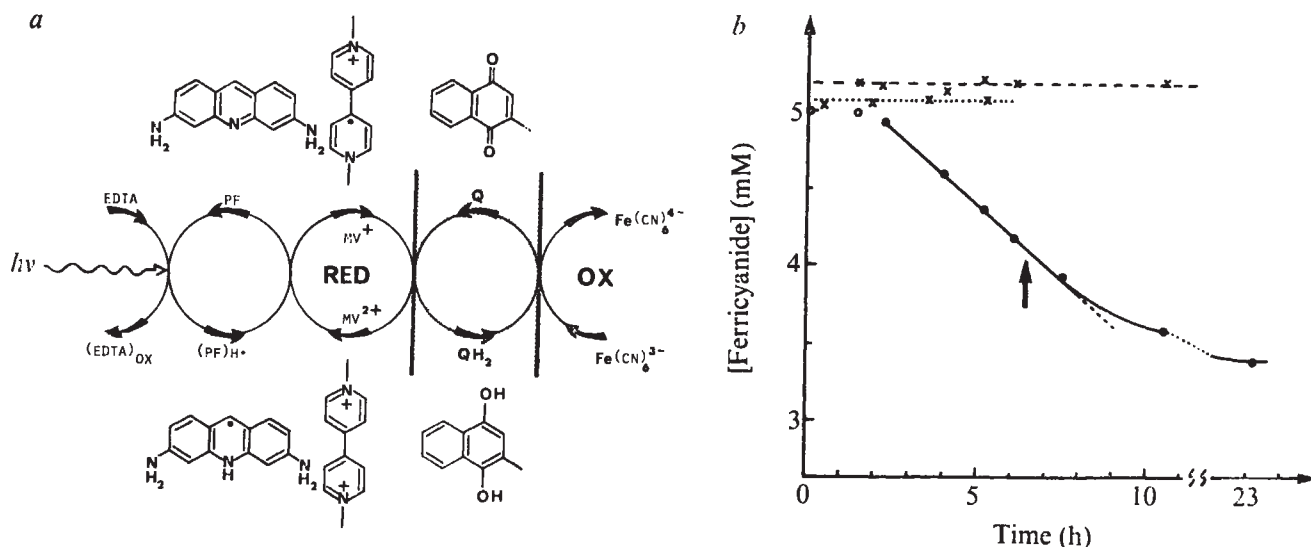


Fig. 1 *a*, Diagrammatic representation of the light coupled transport of electrons across a membrane. The reducing species in the RED aqueous phase at left is the radical cation $MV^{\cdot+}$ produced via proflavine (PF) sensitised photoreduction of methyl viologen MV^{2+} by the electron donor EDTA. The membrane contains vitamin K_3 as carrier molecule and the oxidising agent in OX is ferricyanide. *b*, Typical experiments showing the variation in ferricyanide concentration in the OX phase in three conditions: in the dark (—); under irradiation but with no menadione in the membrane (---); with the complete system shown in Fig. 1*a* (—). In the latter experiment the conditions were: RED: 50 μ M PF + 5 mM MV^{2+} + 10 mM EDTA in 0.1 M phosphate buffer at pH 6.5; membrane: 6.9 mg menadione in 72 mg diphenylether on a Millipore filter; OX: 5 mM potassium ferricyanide in 0.1 M phosphate buffer at pH 7.0. From the linear section of the curve the transport rate is 7.1μ M h^{-1} ; the light was switched off at the point indicated by the arrow; the rest of the curve corresponds to the ferricyanide reduced by the $MV^{\cdot+}$ and the hydroquinone present at the time where the light was switched off.

The efficiency of the photocoupled electron transport (run 10) is comparable with that of the previous chemical redox systems. A problem with this system is, however, that the RED phase slowly bleaches in the course of the experiment but addition of fresh PF restores the original colour. Because bleached solutions continued to effect electron transport, control experiments were performed. The transport rate is the same in the absence of either PF alone (run 11) or of both PF and MV^{2+} (run 12), being half the rate of run 10. Thus the complete system is necessary for highest efficiency and MV^{2+} has no effect in the absence of PF. With EDTA alone, photoactivated electron transport still occurs (run 12), so experiments were conducted under irradiation with an argon laser at 476.5 nm where menadione does not absorb. The transport rate, although similar to that obtained with white light when the whole RED system is present (run 14), now becomes zero with only EDTA in the RED phase (run 15). This confirms that the naphthoquinone carrier may indeed be photo-reduced by EDTA under visible light irradiation with the halogen lamp.

We have demonstrated that electron transport processes across artificial membranes using suitable carrier molecules can be coupled to light irradiation. The search for a more stable photosystem (for example metal cation complexes) may lead to applications in energy storage. On the other hand, coupling of either chemically-induced or photo-activated electron transport to cation (other than proton) symport and anion antiport may be envisaged, providing models for biological systems⁸ as well as new chemical processes and potentially useful transport cells.

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Yes, Kakangari is a unique chondrite

THE Kakangari chondrite seems to be the only representative so far recognised of a new class of chondrites. Mason and Wiik¹ noted a similarity between Kakangari and Mokoia (C3(V)) in bulk chemical composition when expressed on a volatile-free basis, but pointed out that the higher metal content of Kakangari suggests a relationship between it and Renazzo (C2). Recognising that Renazzo contains much less troilite and much more FeO than Kakangari, however, they concluded that Kakangari was a new type of carbonaceous chondrite, more reduced than the others. Graham and Hutchison² emphasised the chemical similarity between Kakangari and the ordinary chondrites, apart from its higher S content, and posed the question, "is Kakangari a unique chondrite?". Using the data of Mason and Wiik¹ as well as their own electron microprobe data, they noted that Kakangari is closer in total Fe content to the L- than the H- group chondrites but that its oxidation state lies between those of the H- and E- chondrites. Clayton *et al.*^{3,4} were able to distinguish Kakangari from the ordinary chondrites on the basis of oxygen isotopes. On a three-isotope diagram, Kakangari plots with the anomalous stony-iron meteorites Bencubbin and Weatherford along an extrapolation of the apparent mass fractionation line defined by the low-temperature hydrous silicate phases of three C2 chondrites. We have now analysed a 17.1-mg piece of Kakangari for 20 elements and can answer Graham and Hutchison's question in the affirmative.

We used the INAA techniques described in previous publications from this laboratory^{5,6}. The standard pot, SP^{7,8}, was used as a standard for most elements. Their concentrations were taken from Perlman and Asaro⁹. The

Table 1 Elemental abundances in the Kakangari meteorite

Element	Concentration (p.p.m., unless otherwise indicated)
Na	6,938 ± 8
Sc	8.70 ± 0.01
Cr	3,590 ± 10
Mn	2,700 ± 40
Fe	21.1 ± 0.1 %
Co	394 ± 1
Cu	170 ± 1
Zn	171 ± 5
Ga	9.7 ± 0.6
As	1.6 ± 0.1
Sb	0.18 ± 0.02
La	0.36 ± 0.02
Ce	1.1 ± 0.1
Sm	0.198 ± 0.009
Eu	0.088 ± 0.003
Yb	0.27 ± 0.02
Hf	0.14 ± 0.03
Os	1.13 ± 0.07
Ir	0.711 ± 0.001
Au	0.206 ± 0.001

concentrations of Os, Ir and Au in Kakangari were determined from chemical standards which had been run in previous irradiations. The specific activities of ^{191}Os , ^{192}Ir and ^{198}Au at the end of the Kakangari irradiation were calculated from their values in previous irradiations by correcting for differences in neutron fluence using ^{46}Sc in SP. The results are shown in Table 1, along with errors based on 1σ counting statistics for sample and standard. Our data for Fe, Na and Mn are within 6–12% of the wet chemical data of Mason and Wiik¹. These small differences probably reflect a small amount of sample heterogeneity. Our Cr content for Kakangari is a factor

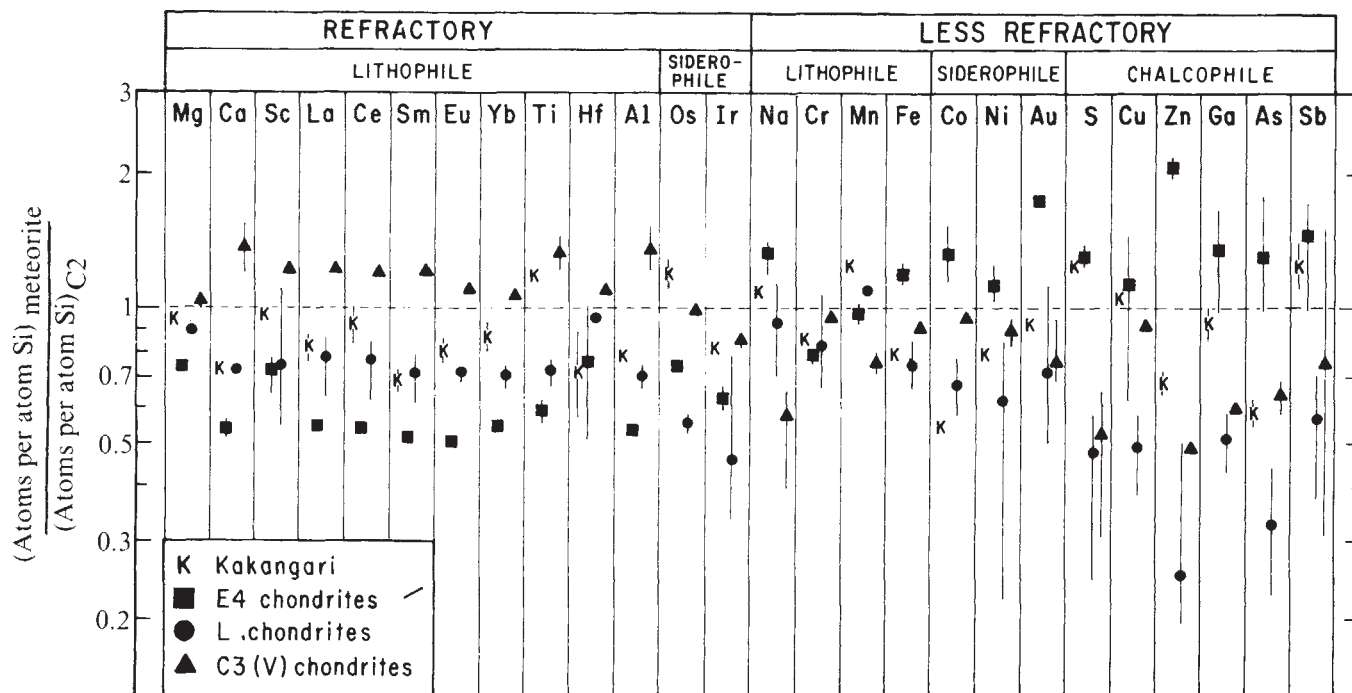
of 2.8 higher and Co a factor of 2 lower, however, than the concentrations reported by Mason and Wiik¹. This may be due to analytical difficulties in the wet chemical determination of low concentrations of these elements. The Ti value of Mason and Wiik¹ may be similarly affected².

In order to compare Kakangari to other meteorite groups, all available elemental concentration data from this work and Mason and Wiik¹ were converted to silicon-normalised atomic abundances. The most similar groups, C2, C3(V), L and E4 chondrites, are represented in Fig. 1, along with Kakangari.

The less refractory lithophile and siderophile elements are at about the same levels in Kakangari and the L-chondrites, but sulphur is enriched in Kakangari by a factor of 2.7 relative to the average L-chondrite and the chalcophile elements Cu, Zn, Ga, As and Sb are enriched by a factor of 2.1 ± 0.2 (1σ standard error). Close inspection of Fig. 1 shows also that the abundances of most refractory elements in Kakangari plot either slightly above their concentration ranges in L-chondrites or at the extreme upper limits of those ranges.

While similarities can be found occasionally in the concentration levels of some elements between Kakangari and either the E4, C2 or C3(V) chondrites, pronounced differences do exist between Kakangari and these other meteorite classes. Kakangari is higher by a factor of 1.47 ± 0.08 than E4 chondrites, lower by a factor of 0.86 ± 0.04 than C2 chondrites and lower by a factor of 0.72 ± 0.04 than C3(V) chondrites in refractory lithophile elements (all uncertainties are 1σ standard errors). Among the less refractory elements, Kakangari is higher than E4, C2 and C3(V) chondrites in Mn abundance, lower than these classes in Co content and intermediate between C2 and C3(V) chondrites in Zn concentration. Note particularly the substantial depletions of Co, Zn and As in Kakangari relative to the C2 chondrites.

Fig. 1 Si-normalised atomic abundances for Kakangari, E4, L and C3(V) chondrites, relative to C2 chondrites. Data for E4, L and C3(V) are shown with their ranges when available. The Kakangari data are shown with their 1σ error bars when the uncertainty is larger than the symbol. The data for E4, L, C2 and C3(V) chondrites are from the following sources: Na: E4, L¹⁰, C2, C3(V)¹¹; Mg, Al, Si, Ca, Ti, Mn, Fe: E4, L¹², C2^{12,13}, C3(V)^{12,14}; S: E4, L, C2, C3(V)¹⁵; Sc, Cr: E4, L, C2¹⁰, C3(V)⁶; Co: E4¹⁶, L¹⁷, C2¹⁰, C3(V)⁶; Ni: E4, L, C2¹⁸, C3(V)¹⁹; Cu: E4¹⁶, L²⁰, C2, C3(V)¹⁰; Zn: E4²¹, L²⁰, C2²², C3(V)²³; Ga: E4¹⁶, L²⁰, C2²⁴, C3(V)²⁵; As: E4¹⁶, L, C2, C3(V)¹⁷; Sb: E4¹⁶, L¹⁷, C2²², C3(V)¹⁹; La, Ce, Sm, Eu, Yb: E4, C2, C3(V)²⁵, L²⁶; Hf: E4²⁷, L, C2²⁸, C3(V)²⁹; Os: E4, L, C2, C3(V)³⁰; Ir: E4, L³¹, C2²², C3(V)¹⁹; Au: E4³², L²⁰, C2²², C3(V)¹⁹.



Our trace element data thus confirm the conclusion of Mason and Wiik¹ and answer the question posed by Graham and Hutchison². Kakangari is a unique chondrite in that its bulk chemical composition does not match that of any single known class of chondrites.

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surely present that could carry food to it². Evidence supporting any of these views is unreliable. On the Ross Ice Shelf, samples taken through cracks 22 and 28 km from the open sea and in a newly calved area 8 km from the former ice front yielded rich benthic faunas but no floras^{3,4}. Plant life was presumably restricted by the lack of light coming through the ice. Some of the more common species of animals at nearby McMurdo Sound were not found in the samples, indicating that there was a possibility that conditions under the ice precluded them. Such differences, however, are common in Antarctic shallow-water areas, and can be due to local substrate differences, recent grounding of ice, or sampling bias dependent on the size and penetration depth of the sampler⁵. The Ross Ice Shelf locations are so close to the open ocean that typical benthic communities might live there. At George VI Sound, marine fish whose stomachs contained several species of invertebrates were caught through a proglacial lake adjacent to the ice shelf 100 km from the nearest open water⁶. These animals were interpreted as evidence that a marine biome occurs under

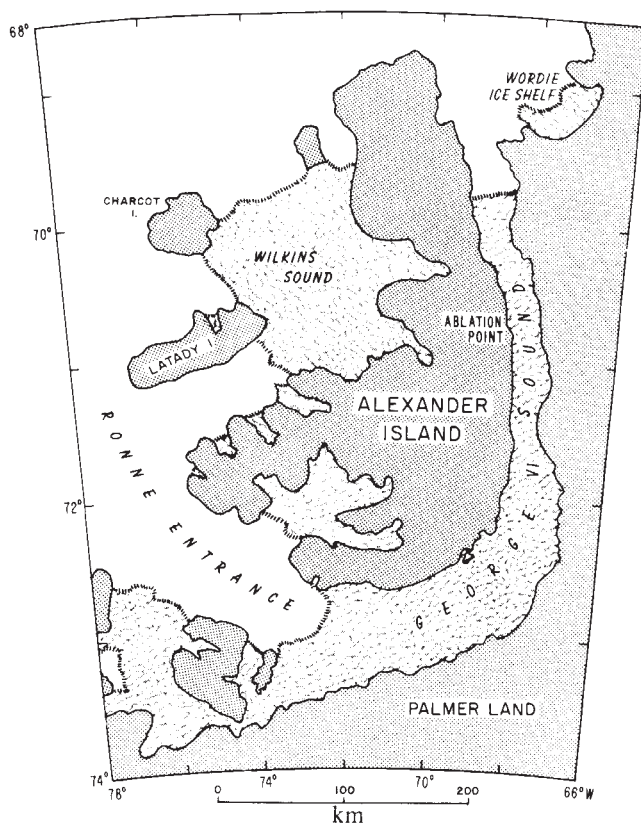


Fig. 1 George VI Sound region and Ablation Valley near the base of the Antarctic Peninsula. Ice shelves are shown in light stipple. Map from ref. 7.

the entire shelf⁶. However, we describe here a microbiota from the George VI Sound occurrence that indicates that the fauna there may also be unique because it resides in a situation not typical of the entire ice shelf. Available evidence thus does not support any opinion.

George VI Sound is approximately 475 km long and 20 to 60 km wide, and is open on both ends to the Bellingshausen Sea (Fig. 1). It is covered by permanent ice varying between 100 and 500 m thick⁷, but there are a few holes and cracks in it through which samples may be obtained. Mud samples collected in the proglacial lake at Ablation Valley (Fig. 1), where the fish were caught, yielded an abundant microbiota. As described by Heywood and Light⁸, the lake, 5 by 4 km in size, lies adjacent to the sound and is intruded for 3.4 km

Microbiota under Antarctic ice shelves

WHETHER or not life can exist far from the open sea beneath the permanent ice shelves of Antarctica is not known, but there are three opinions. (1) A biota cannot exist at all because of the absence of surface primary and secondary productivity¹. (2) A biota exists but with specialisations unique to life in the absence of surface productivity and in isolation under an ice shelf¹. (3) A typical Antarctic biota exists under any Antarctic ice shelf because currents are

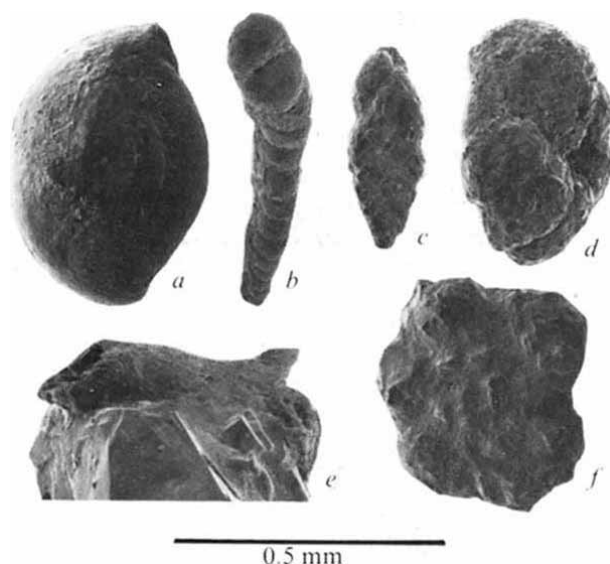
Table 1 Microbiota from two bottom samples taken in a proglacial lake at Ablation Valley, George VI Sound, Antarctica

Diatoms		
Sample from 47-m freshwater		
<i>Achnanthes brevipes</i> Agardh 1824	500	100
Samples from 68.5-m seawater		
<i>Achnanthes brevipes</i> Agardh 1824	1	0.3
<i>Amphora ovalis</i> (Kützing) Kützing 1844	1	0.3
<i>Amphora</i> sp.	25	5.0
<i>Diploneis</i> spp.	5	1.0
<i>Gyrosigma wansbeckii</i> var. <i>subsalina</i> (H. Peragallo) Cleve 1894	1	0.3
<i>Melosira tcherniai</i> Manguin 1960	88	18.0
<i>Navicula directa</i> (Wm. Smith) Ralfs in Pritchard 1861	2	4.0
<i>Synedra affinis</i> Kützing 1844	42	8.0
<i>Synedra</i> spp.	327	65.0
<i>Trigonium arcticum</i> (Brightwell) Cleve 1868	5	1.0
<i>Tropidoneis lepidoptera</i> (Gregory) Cleve 1894	3	0.6
	500	≈ 100
Foraminifera		
Samples from 68.5-m seawater		
<i>Conotrochammina rugosa</i> (Parr)		
<i>Cribrostomoides wiesneri</i> (Parr)		
<i>Haplophragmoides canariensis</i> (d'Orbigny)		
<i>Miliammina arenacea</i> (Chapman)		
<i>Pseudobolivina antarctica</i> Wiesner		
<i>Reophax</i> sp.		
<i>Textularia wiesneri</i> Earland		
<i>Tolypammina vagans</i> (Brady)		
Gen. et sp. indet. no. 1		
Gen. et sp. indet. no. 2		

by the ice shelf. It is covered by ice, 2.5–4.5 m thick, throughout the year. Light can penetrate this thickness of ice. The lake is over 117 m deep, but only below 66.25 m is there marine water. Tidal fluctuations in the marine layer show that it is connected with the seawater of the sound⁸.

The microbiota (Table 1) includes 10 species of foraminifera (Fig. 2) and at least 11 species of diatoms found in a marine sample at 68.5 m and one diatom species from a freshwater sample at 47 m. Mixing of the biotas from these water layers was not apparent in our samples. All foraminifera and diatom species are benthic. The diatoms and foraminifera are nearly perfectly preserved and quite abundant; most of the diatoms still retain some pigment.

Fig. 2 Foraminifera from the marine layer at Ablation Valley, George VI Sound. Scanning electron micrographs. *a*, *Miliammina arenacea*. *b*, *Textularia wiesneri*. *c*, *Pseudobolivina antarctica*. *d*, *Conotrochammina rugosa*. *e*, Gen. et sp. indet. Form attached to rock fragment. *f*, *Conotrochammina rugosa*.



The specimens seem to have been alive or recently dead when collected. No other microorganisms possessing hard parts were found. We have found all of the species, including the fish and invertebrates, farther north in ice-free waters along the Antarctic Peninsula^{5,9}. Centric and pennate diatoms have also been found in seawater present in crevasses on the Ross Ice Shelf 550 km from the open Ross Sea¹⁰.

Our results reinforce previous conclusions⁶ that an abundant and diverse Antarctic marine biota lives at Ablation Valley. Heywood and Light⁶ concluded that the productivity they recorded for the freshwater layer was too low to support fish populations. The abundance of marine diatoms from lower levels, however, indicates that there is also marine primary productivity, probably sufficient to sustain higher trophic levels.

The microbiota we record from the proglacial lake can be interpreted in either of two ways: (1) it may indicate that there is indeed a diverse and rich biome under the George VI Ice Shelf as postulated^{2,6}, in which case it would have to be supported by a food supply carried there by currents² passing through the sound; or, (2) it may mean that the biota at Ablation Valley is atypical of the sound as a whole because the marine primary productivity may support a biological oasis where light penetrates through the thin ice cover of the proglacial lake. Although some diatoms perhaps could live heterotrophically under a thick ice cover, we conclude that the second possibility cannot be eliminated. Thus, in spite of a few occurrences near ice fronts and in the unique situation at Ablation Valley, there is still no unequivocal evidence of what a sub-ice shelf biome might be like.

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First Devonian platyspermic seed and its implications in gymnosperm evolution

IN the course of an investigation of fossil plants from the late Devonian rocks of Kiltorcan in southern Ireland obtained by re-excavation of the site earlier this year, we have discovered several seeds with features which contribute to the understanding of early gymnosperm evolution. The fossil flora of Kiltorcan has been known for more than a century¹, and includes the well-known and widespread late Devonian genera *Archaeopteris* and *Cyclostigma*. In addition, Johnson² briefly described and illustrated several oval bodies which he regarded as seeds, and to which he gave the

name *Spermolithus devonicus*. His figures were at a small scale and rather uninformative, and most later workers discussing early evidence of seed plants have either been unaware of his claim, or make no reference to it. The only reference to *Spermolithus* known to us is Arnold's³ justifiable comment that "whether it is a seed or spore case is unknown to us". In spite of the limitations of their state of preservation, we believe that the seeds we have found represent not only the earliest seed plants known from the British Isles, but also the earliest seeds with apparently platyspermic symmetry. In view of their closeness in age to the earliest generally accepted seed, *Archaeosperma*, of radiospermic character, we believe that this favours a more or less synchronous origin of radiospermic and platyspermic gymnosperms. This suggests that these two seed lineages have been separate since their inception, rather than having had a single evolutionary origin followed by rapid divergence within the Carboniferous.

Knowledge of early seed plants has grown significantly in the past decade¹⁻⁷, and has an important bearing on the interpretation of their early evolution. The most convincing record of a seed of Devonian age is that of *Archaeosperma* Pettitt and Beck⁸ from the North American Famennian. This seed, although a compression, has been shown to have at least four integumentary lobes making it unmistakably radiospermic. Long^{1,8,9}, in his extensive researches into early Carboniferous (Tournaisian) seed plants, has found both platyspermic and radiospermic seeds with well preserved structure. He has suggested⁴ that the platyspermic condition evolved from radial ovules with only two integument lobes, showing that in the early stages of platyspermy they retain the radial symmetry at the nucellar apex. This

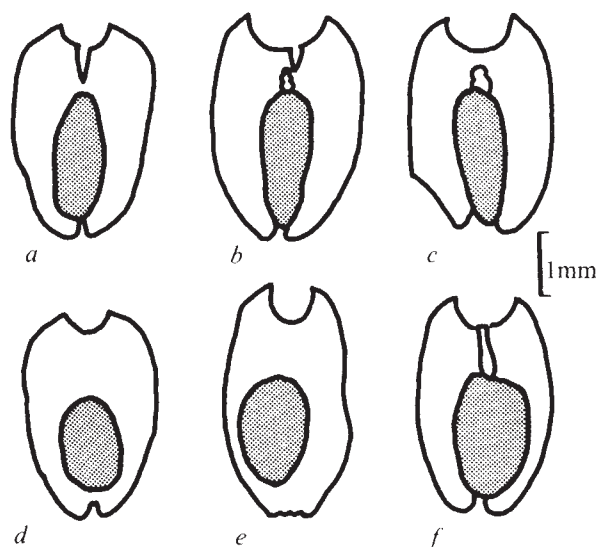


Fig. 2 Outline drawings of six specimens of seeds, cf. *Spermolithus devonicus* Johnson drawn from photographs. The scale bar is 1 mm. These show the consistent presence of the apical embayment with lateral lobes. The megaspores (stippled) show greater variation in size and shape than the enclosing structure, a characteristic feature of seed-megaspores in Palaeozoic seeds.

would be consistent with a single origin for seed plants in a radiospermic Devonian ancestor such as *Archaeosperma*. Other authors¹⁰⁻¹¹ acknowledge the possibility of a polyphyletic origin of gymnosperms. Our discovery supports such a hypothesis.

There are several records of seed-like bodies from the late Devonian or basal Carboniferous¹²⁻¹⁵ of which *Condrusia minor* Stockmans¹³ and *Moresnetia zaleskyi* Ananiev¹⁶ are of particular interest. These have a distinct bicornute shape, implying platyspermy, but far too little is known about these bodies even to state definitely that they are seeds.

The discovery of the assemblage of seeds at Kiltorcan makes some contribution to this controversy of seed plant origins. The Kiltorcan fossil plant material is in an unusual, and in some ways unsatisfactory, state of preservation. The plants were evidently initially preserved as coalified compression fossils¹⁷ in which the coaly substance was subsequently entirely replaced by a chloritoid mineral¹⁸. Although the resulting fossils make striking museum specimens, they are not amenable to oxidative maceration, which is the normal means of making microscopic (cuticle) preparations of fossil plant compressions. Immersion of the fossils in xylene and observation under strong incident lighting, however, reveals limited information on their internal organisation (Fig. 1).

It is critical to our case to establish that our fossils (Figs 1 and 2) are not merely seeds but that they are of platyspermic organisation. The possibility of discriminating reliably between radiospermic and platyspermic seeds, even when compressed in fossilisation, was first advanced convincingly by Arber¹⁹. The 30 seeds showing more or less intact outline that we recovered from Kiltorcan show a uniformity of size and shape consistent with plant propagules shed in a mature condition; in particular, they regularly show an eccentrically-placed dark oval body (stippled in Fig. 2) close to a small notch and a larger, presumably apical, embayment with two associated lobes. By analogy with better preserved seeds, we can interpret the embayment as the site of the micropyle (as in the widespread Carboniferous platyspermic seed *Samaropsis*) and the dark inner body as the megaspore (as in *Menaesperma*²⁰ where maceration of the inner body shows it to be a megaspore surrounded by the nucellus), and in some of our seeds

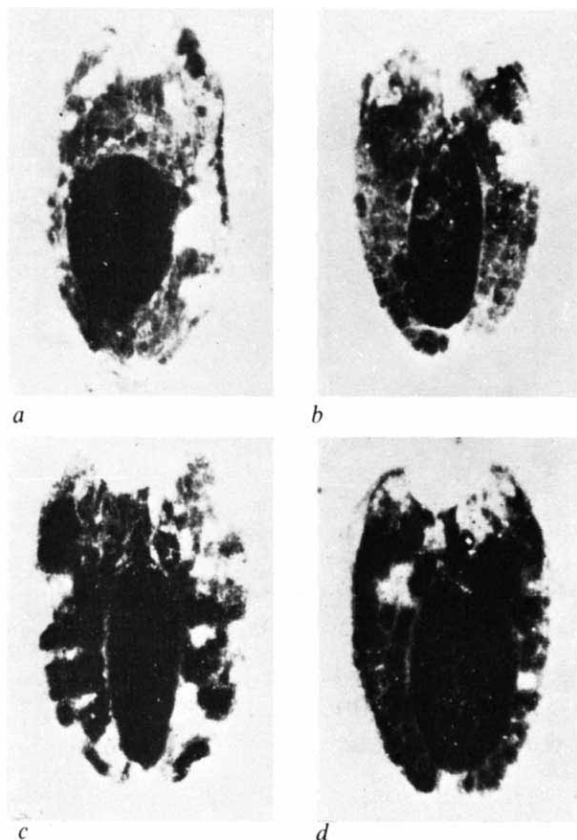


Fig. 1 Four seeds, cf. *Spermolithus devonicus* Johnson preserved as chloritised compressions from the Upper Devonian Kiltorcan Beds, Kiltorcan "Old Quarry", County Kilkenny, Eire ($\times 14$, photographed under xylene). The assumed apical (micropylar) embayment is uppermost in each case; a basal notch is discernible, for example in (d). The dark central structure is interpreted as a megaspore. A possible nucellar apical expansion (or micropylar canal infill) is discernible in (c) and (d).

there is an indication of what may be a free nucellar apex or micropylar canal (Fig. 2b, c, and f). This interpretation of the inner body is supported by its variation in both size and shape (Figs 1 and 2). Other workers^{20,21} have demonstrated such variation in several well-preserved fossil seeds. The consistent occurrence of the apical lobes and intervening embayment rules out the possibility that we are simply seeing a random fracture plane passing through a broad micropylar opening (such as might occur in a compression state of the radiospermic *Stamnostoma*). We believe that the generally cordate form of our seeds aligns them with typical Carboniferous members of the genus *Samaropsis*, particularly *S. crassus* and *S. subacuta*¹⁹. On this interpretation, these Kiltorcan seeds are regarded as lying with their major transverse axis in the bedding plane as they became buried.

Provisionally, we assign our seeds to Johnson's species *Spermolithus devonicus*. His seeds, so far as can be judged from his photographs and brief description, show similar features to ours in appearing to have the oval shape, apical embayment, a central body and approximately similar size. A search for Johnson's original figured (type) material in museum and university collections in Dublin and elsewhere has been unsuccessful.

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Mouth gaping as an effective thermoregulatory device in alligators

GAPING is a characteristic activity of several species of crocodilians in which the animal lies ashore with its mouth held open for prolonged periods¹⁻⁵. It has stirred the interest of humans for millennia: gaping is depicted in Egyptian papyrus and was mentioned in the writings of Herodotus and Pliny. Today interest continues in gaping and other aspects of behaviour in these large reptiles, the last of the subclass Archosauria, the group of reptiles that included the dinosaurs^{1,5}. Gaping is a complex habit that involves variable degrees of

mouth closure and sometimes includes rhythmic gular movements. Behavioural observations have suggested that gaping is a thermoregulatory response^{3,4} but until now physiological studies have been inconclusive. Initial reports⁶ stated that gaping was effective in cooling young Nile crocodiles (*Crocodylus niloticus*) but a recent experiment on *Caiman crocodilus* indicated that this behaviour had no thermoregulatory significance⁷. Here we present evidence from controlled laboratory and field experiments that gaping is an effective mechanism for reducing head temperatures of alligators (*Alligator mississippiensis*). Gaping reduced the rate of heat gain by the heads of small and large alligators when these animals were exposed to high light radiation loads at moderate air temperatures. The magnitude of this effect increased with size. Gaping had little effect on body temperature and did not reduce the final equilibrium temperature of the head.

Head, palate and body temperatures were measured (Cu-Co thermocouples) in three alligators under controlled conditions in an environmental chamber. Alligators were anaesthetised with ketamine hydrochloride⁸ and a 38-gauge thermocouple inserted in the head to a depth of 1.5 cm in the upper temporal fossa, anterior to the adductor externus profundus muscle. Palate temperature was measured with a 38-gauge thermocouple placed under the skin below the palatal fenestra and body temperature was measured with a 24-gauge thermocouple inserted through the cloaca into the colon to a depth of 10 cm. Incisions were sealed with glue (Eastman 910) and animals were allowed to recover for a period of 24 h before being tested. Each alligator was tested twice, once with its mouth closed and once with its mouth propped open at an angle of 35° to simulate the gaping posture. Alligators were placed in an environmental chamber at an air temperature in the range of 20 to 23 °C and heated by radiation and conduction until body temperature reached equilibrium. Sodium vapour lights were used to give high visible flux, and floor temperatures were controlled at an elevated level. Wind speed was 190 cm s⁻¹ and radiation absorbed by the alligators was 0.76 cal cm⁻² min⁻¹, about that of mid-morning on a clear day.

In all three cases the heads of alligators with their mouths propped open heated at a lower rate than those of alligators with their mouths closed (Table 1). This was indicated by the determination of time constants. Time constants for the equilibrium of head temperature for the open mouth condition (τ_o) were greater than those for the mouth closed state (τ_c), ranging from 1.4 times greater for the smallest animal to 2.1 for the largest. The time constants for the rate of heating of the body (that is, cloaca) with the mouth open were similar to those with the mouth closed.

We conducted an additional experiment on a large adult (total length 2.92 m, estimated weight 100 kg) in the field. The animal was captured during October, 1975 at the Savannah River Ecology Laboratory (Aiken, South Carolina). Thermocouples were implanted as described previously and after a 24-h recovery period the alligator was placed in the sun with its mouth propped open (20° gape) at mid-morning on a clear day. The animal was not heated to its equilibrium temperature since this temperature would have been above its lethal limit. After 160 min, head temperature had risen from 23.2 to 32.7 °C and body temperature had risen from 23.4 to 26.3 °C. The next day the alligator was heated again in similar environmental conditions, but with its mouth closed. Now after 160 min head temperature went from 23.1 to 35.0 °C and body temperature rose from 23.1 to 25.2 °C. Body temperature rose 2.9 °C on the first day and 2.1 °C on the second day. This indicated that although environmental conditions seemed similar, this alligator actually had a lower net heat input to the body during the mouth closed test. Despite this fact, head temperature rose 11.9 °C with the mouth closed as compared to 9.5 °C with the mouth open. This strongly suggests that gaping was important in reducing the rate of heat gain to the head region. Thus it seems that the effect of gaping on a 100-kg alligator was similar to that on animals ranging in size from

Table 1 Temperatures and time constants for alligators heated under controlled environmental conditions with their mouths open and closed

Weight (g)	Length (cm)	Location of thermocouple	Temperatures (°C)				Time constants*		
			Mouth open		Mouth closed		τ_o	τ_c	τ_o/τ_c
			Initial	Final	Initial	Final			
2,243	97.0	Head	17.4	30.2	17.4	30.4	50	35	1.4
		Body	15.9	30.7	16.8	30.6	45	40	1.1
		Palate	16.6	29.9	17.2	30.3	45	30	1.5
4,698	125.0	Head	22.4	36.2	22.2	35.9	48	29	1.7
		Body	22.2	36.4	21.8	35.3	44	41	1.1
		Palate	21.9	—	21.3	—	—	—	—
14,512	170.0	Head	23.9	32.2	23.5	32.8	145	68	2.1
		Body	18.6	31.5	19.4	31.6	144	128	1.1
		Palate	15.4	27.4	19.2	29.8	90	98	0.9

* τ_o , Time constant for mouth open; τ_c , time constant for mouth closed. A thermal time constant for a linear first-order system is the time required for the temperature of an object to travel $(1 - e^{-1})$ or 63.2% of the way to the applied temperature. The smaller the time constant the faster the temperature change. A standard heating- or cooling-curve experiment is an example of a linear first-order system. In this case body temperature changes as a function of time: $T = T_f - (T_o - T_f)e^{-t/\tau}$ where T is body temperature (°C), T_o is initial body temperature (°C), T_f is final body temperature (°C) and t is elapsed time (min). The parameter τ is the time constant. An alligator coming into equilibrium with heat sources and sinks in a complex environment can be approximated as a linear first-order system^{9,10}.

2.2 to 14.5 kg in weight, since rates of heat gain by the head were reduced in all cases when gaping was simulated. Gaping was an effective thermoregulatory mechanism in the environmental conditions tested in this study.

These results are expected from energy budget theory^{11,12}. Exposure of the moist mucous membranes of the mouth during gaping would result in evaporative cooling. The rate of cooling would increase at low relative humidity and in the presence of a breeze. The mechanisms by which heat is transferred from the brain region to the mouth where it is dissipated during cooling are not known at present. Heat may be transferred by direct conduction through the bony skull and/or by blood circulation. Transfer of heat may also be occurring between the head and body by way of the circulatory system as has been demonstrated for *C. johnstoni*¹³. However, head-body temperature differences in reptiles can result from physical factors independent of the animal's control¹⁴. Therefore, definitive comments on mechanisms of heat transfer within the alligator cannot be made until further investigations of vascular patterns, conduction pathways and the mechanics of heat flow within the head have been carried out.

Though physiological studies under controlled conditions clearly indicate that gaping is a potentially useful mechanism for controlling heat gain of the head region in these animals, further studies are needed to define the importance of gaping in wild alligators. We expect that gaping would be most effective in spring, autumn and possibly winter in portions of the alligator's range in which it does not hibernate. These are times when alligators are exposed to cool water and air temperatures and should rely extensively on basking to raise their body temperature above ambient¹⁵. Gaping would reduce heat gain by the alligator's head and allow the animal to continue basking to allow its body temperature to continue to rise towards preferred levels. Then the alligator could retreat to the water to avoid over-heating. Gaping would be most effective in large alligators since their high heat storage capacity would require that they bask for long periods of time in order to raise their body temperature a few degrees.

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22-kHz calls by isolated rats

CALLS of about 22 kHz occur during two kinds of social interaction among rats, *Rattus norvegicus*: they are produced by submissive animals in agonistic encounters¹ and by males after ejaculation^{2,3}. I have now discovered that calls of similar structure regularly occur without social stimulation and in a daily pattern controlled by the light-dark cycle. Such 'spontaneous' 22-kHz calls may help in the maintenance and development of other behaviour patterns.

The experiments reported here mainly involved round-the-clock recording of calls made by socially isolated, adult Wistar rats. Before observation each animal was entrained for at least 1 month to the light-dark schedule under which it was observed. During observation each animal was kept alone in an unused, quiet storeroom. A daily check was made on food, water and the operation of the lights, and vaginal smears were taken from the females. Calls were monitored with a bat-detector tuned to 23 kHz. This receiver produces an audible output signal in response to an ultrasonic input signal within 2-3 kHz of the frequency to which the receiver is tuned. Its output was continuously recorded on slow-moving magnetic tape which was periodically reviewed at a higher speed. Bouts of calls (groups of calls with a maximum interval between calls of 0.1 h) were replayed at the original speed, and the amount of time

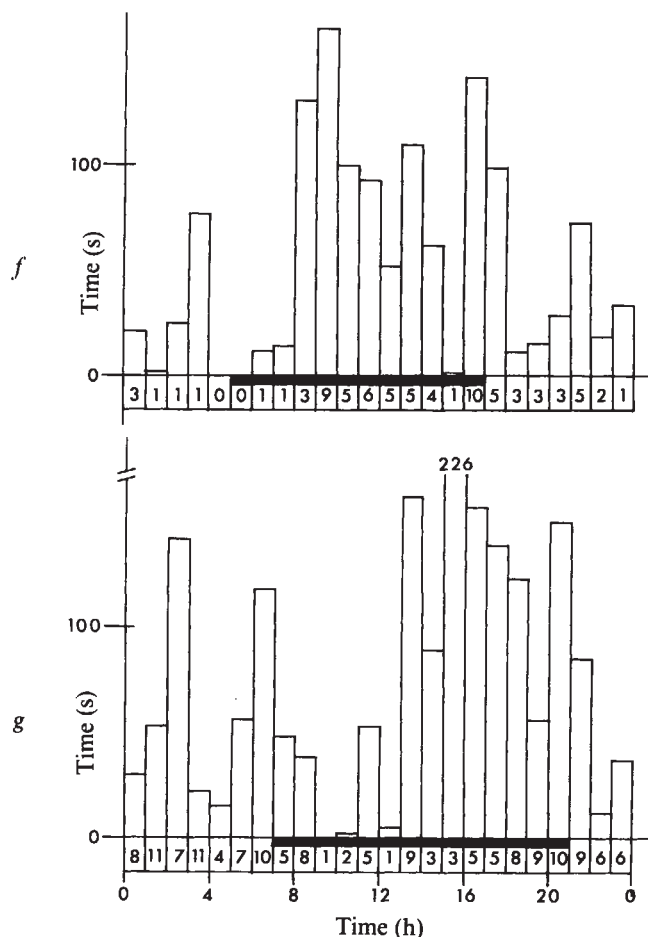
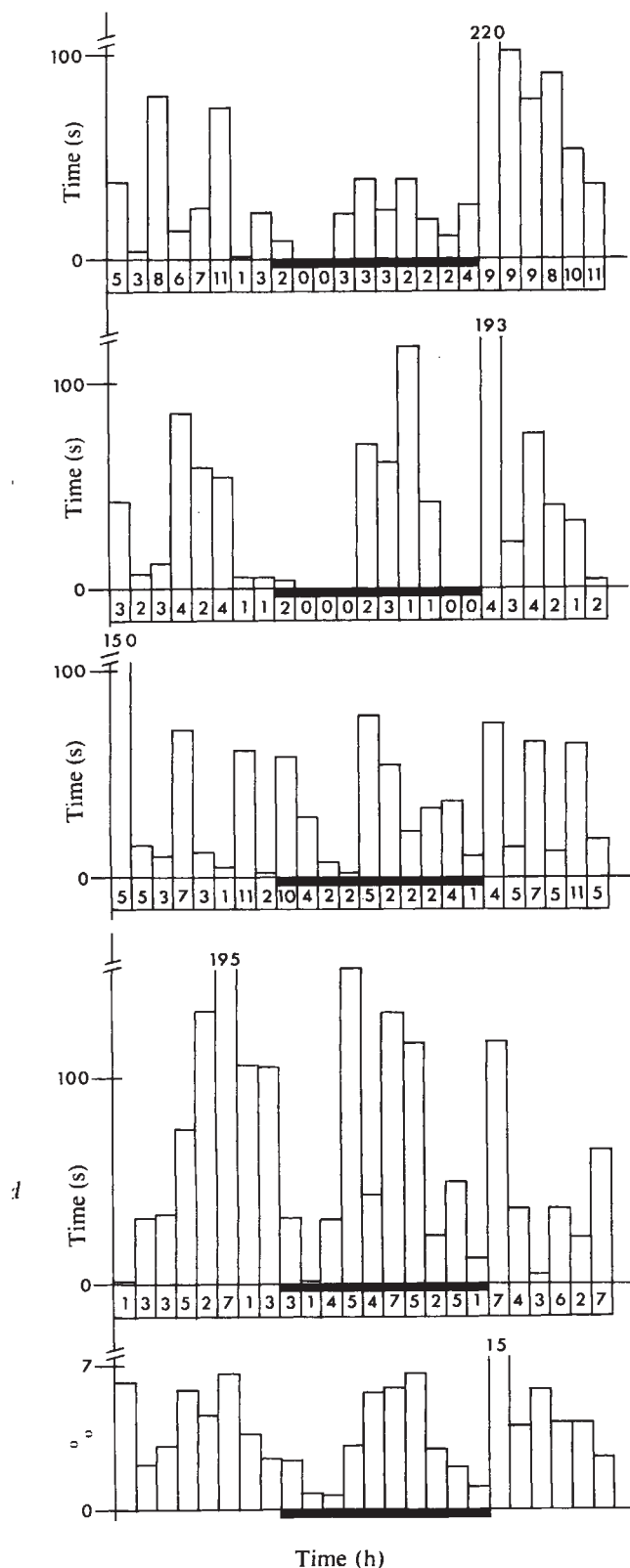


Fig. 1 Distributions of calling by six rats. Each column represents the total amount of calling(s) in that hour, summed over all days of observation. In hours in which this amount exceeded the maximum value on the ordinate, the column is topped with a number which is the amount observed in that hour. The total number of bouts in each hour and the hours of darkness are indicated below the abscissa. Hour 0 was midnight for all animals. *a*, An intact female with a 5-d oestrous cycle, observed for 20 d. *b*, An intact female with a 4-d oestrous cycle, observed for 12 d. *c*, An ovariectomised female, observed for 30 d. *d*, A male, observed for 7 d. *e*, Mean distribution of calling by animals *a* to *d* above, which were on the same 14:10 light-dark cycle; each column is the mean of the four individuals when calling for that hour is expressed as a percentage of the individual's total amount of calling. *f*, An intact female with a 4-d oestrous cycle, observed for 14 d on a 12:12 light-dark cycle. *g*, An intact female with no regular oestrous cycle, observed for 27 d on a 10:14 light-dark cycle.

spent calling was manually recorded on a cumulative timer. Sometimes the animals were also monitored with a second bat-detector tuned to 25 kHz, the output of which was recorded on a second channel of the tape recorder. This expanded the frequency range over which calls could be detected, and provided a convenient method for estimating the frequency of a call. The frequency of almost all calls was between 25 and 22 kHz. For consistency with other authors^{2,3}, these sounds are referred to as '22-kHz calls'.

Animals usually produced a number of calling bouts in each 24-h period. Within and between animals these bouts

varied in duration (from less than a second to several minutes) and in the times when they occurred. Over a period of days, however, the distribution of time spent calling followed a pattern which was consistent for all animals regardless of their hormonal or environmental conditions (Fig. 1): the amount of calling was least in the early hours of darkness (not always the first hour), rose to a peak in the middle of the dark period, and declined towards the end of this period. When there was a 10-h dark period the decline ended abruptly in the first hour of the light period; with longer dark periods the decline ended in the last hour of darkness. There was much individual variation in calling through the rest of the light period, although there was a tendency for calling to decline gradually and then to peak again several hours before dark. Distributions *a*–*d* are highly similar ($W=0.581$, Kendall coefficient of concordance; $P<0.01$) between the onset of darkness and the

peak of calling at its offset. The similarity is not as great for the entire 24-h period ($W=0.377$, $0.10>P>0.05$). The distribution of calling bouts is similar to the distribution of time spent calling (Fig. 2).

The lighting schedules of different animals were such that calling by some animals was least at hours (in real time) when calling by other animals was high. For example, in hour 18–19 (Fig. 1) calling was high for the four animals in *e*, very low for animal *f* and intermediate for animal *g*. This indicates that environmental factors other than the light-dark cycle did not influence calling.

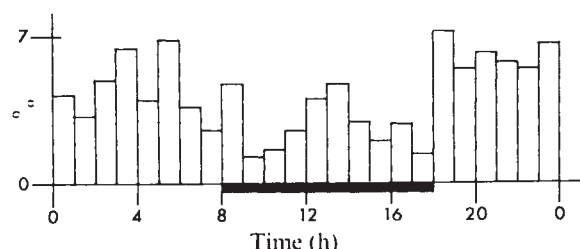


Fig. 2 Distribution of numbers of calling bouts for the four animals on the 14:10 light-dark cycle (Fig. 1, *a-d*). Each column represents the mean of the four individuals when the number of bouts in that hour is expressed as a percentage of the individual's total number of bouts.

Because stressful stimulation, such as electric shock or rough handling may also elicit calling², the stress of social isolation might have been responsible for the 'spontaneous' calls. But calling frequently occurs in lactating females caged with their young, in animals individually caged next to each other, and in colonies of both laboratory and wild rats (unpublished findings). During most of these latter observations there were no overt signs of agonistic or sexual behaviour, which suggests that 'spontaneous' calling is normal for grouped as well as isolated rats.

'Spontaneous' calling is evidently associated with inactivity and rest. Activity and feeding of rats is intense in the early hours of darkness, which is when calling is least. Furthermore, a number of authors report that feeding and activity then subside and later rise to a second peak a few hours before the onset of light¹⁻³, a common pattern among nocturnal mammals. This is the obverse of the pattern in 'spontaneous' calling, the peaks of which could all be associated with times of rest when animals are preparing for, or recovering from, activity peaks during darkness. 'Spontaneous' calls may be associated with sleep. On some occasions I simultaneously observed the behaviour and calling of lactating females with their litters. Nearly always the female was apparently asleep when calling occurred. Furthermore, the EEG pattern during post-ejaculatory calling is identical to the pattern during sleep³, and the overt behaviour of calling animals in social contexts is subdued and 'sleep-like'¹⁻³.

22-kHz calls in social situations follow interaction between two animals; calls by one animal seem to help pacify the other¹⁻³. Since 'spontaneous' calls are not part of any such interaction, their function is not immediately apparent. Nevertheless, it is unlikely that these calls are non-functional because they are so common, because they do not occur randomly, and because they are continually produced even though they could endanger the caller in natural conditions. Many predators are probably sensitive to these calls⁹, which are generally quite intense; post-ejaculatory calls can be as intense as 80 dB SPL². If calls promote inactivity in conspecifics regardless of the context, their emission during inactivity could synchronise the behaviour of animals within a colony. Synchrony in the onset and ending of activity in rat colonies has often been observed,

and Calhoun suggested that calls during activity may be involved. The present results suggest that calls during inactivity may be responsible. It is also interesting to speculate whether calls by lactating females play any part in forming an association in the pups between the sounds and an inactive or relaxed state.

Bell has suggested that 22-kHz calls indicate a state of decreased 'arousal' and may produce a similar state in other rats¹⁰. The present interpretation of 'spontaneous' 22-kHz calls is consistent with both suggestions. Bell further suggests that current hypotheses about functions of rodent ultrasounds may be unnecessarily complicated because they are based on information about the structure and behavioural context of the calls. He advocates research into the physiological state of the calling animal and into the effects of calls on conspecifics. My results indicate that this approach will be necessary in future studies of 22-kHz calls in rats.

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Specific alterations in phosphorylation of cytosol proteins from differentiating neuroblastoma cells grown in culture

THERE have been a number of reports¹⁻¹⁰ which indicate that an experimentally induced elevation in the intracellular level of adenosine-3',5'-monophosphate (cyclic AMP) in mouse neuroblastoma cells induces and increases the expression of many differentiated functions that are characteristic of the mature neurone. The irreversibility of cyclic AMP-induced "differentiation"³ was attributed to the increased level of proteins which bind cyclic AMP thereby protecting the newly synthesised cyclic nucleotide from hydrolysis^{11,12}. However, the mechanisms by which cyclic AMP initiates the observed morphological¹⁻³ and biochemical³⁻¹⁰ alterations remain unclear since the activity of cyclic AMP-dependent protein kinase did not change in differentiated cells¹³. We have investigated the phosphorylation of specific proteins in control and 'differentiated' neuroblastoma cells. We report here that the cyclic AMP-dependent phosphorylation of a specific protein in the cytosol of 'differentiated' cells increased while the cyclic AMP-independent phosphorylation of another protein in this cell fraction decreased. These selective alterations may

represent intermediate steps in a series of events which ultimately lead to the expression of differentiated functions.

Cells of the previously defined³ clone NBP₂ were cultured and maintained as previously described¹. Differentiation was induced by treating the cells for 3 days with 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO20-1724)¹¹, a rather specific inhibitor of cyclic AMP phosphodiesterase^{14,18}. Control and treated cells were homogenised in 0.25 M sucrose containing 0.05 M Tris-HCl buffer (pH 7.4), 3 mM MgCl₂ and 4 mM 2-mercaptoethanol^{11,12}. The cytosol (supernatant of a 9×10^6 g min centrifugation) was diluted in homogenising buffer to contain 2 mg protein ml⁻¹ (Lowry method) and stored at the temperature of dry ice until assayed. Endogenous protein phosphorylation was assayed as previously described¹⁶. Each of the samples studied was also tested for ³H-cyclic AMP binding. Binding was found to be two-fold higher in 'differentiated' cells compared to the controls, as previously reported^{11,12}.

The cytosol of neuroblastoma cells revealed the presence of over 40 protein bands after the proteins of this fraction were electrophoresed in SDS gels. No differences in staining patterns were observed between samples obtained from control cells, cells that had been treated with RO20-1724, and cytosol fractions treated with the drug *in vitro* (Fig. 1). Incubation with ATP before electrophoresis also did not affect the patterns. Protein bands that incorporated ³²P-phosphate upon incubation were designated according to their apparent molecular weight (Fig. 1, autoradiogram). Five of these protein components (bands 55, 59, 72, 88, and 97) incorporated considerably more ³²P-phosphate than the other phosphorylated bands (Figs 1 and 2). Of these five major bands, the phosphorylation of one band (band 97) was not affected by exogenously added cyclic AMP. Phosphorylation of this band decreased approximately two-fold after differentiation (Table 1). The phosphorylation of the other major bands (bands 55, 59, 72 and 88) was stimulated by cyclic AMP. Addition of cyclic AMP (5 μ M) stimulated phosphorylation 128–208% depending on the protein band; the highest stimulation was noted at band 59. Compared with control cells, these four major bands of the differentiated cells showed a marked increase in phosphorylation which varied from 1.2- to 2-fold. The greatest increase occurred in band 59 (Table 1). The percentage stimulation of phosphorylation caused by the addition of exogenous cyclic AMP to samples from differentiated cells was only slightly higher than in control cells. The concentration of cyclic AMP required for half-maximal stimulation (about 0.1 μ M) was found to be the same in both treated and untreated cells.

In order to test whether residual drug levels in 'differentiated' cells were responsible for the changes in endogenous phosphorylative activity observed, the cytosol

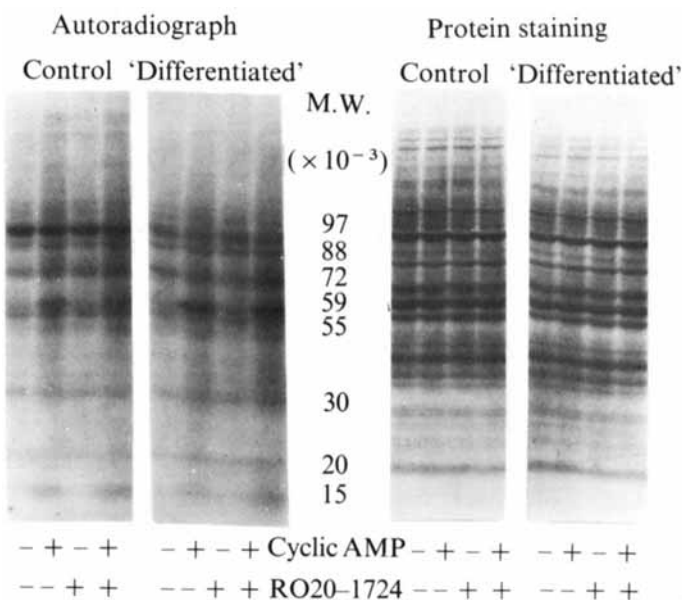


Fig. 1 Identification of endogenously phosphorylated proteins in the cytosol of control and 'differentiated' neuroblastoma cells. Standard assays of endogenous phosphorylation²⁰ were conducted by preincubating aliquots from the cytosol for 5 min at 30 °C. The reaction was initiated by the addition of γ -³²P-ATP (ICN, Irvine, California; diluted with non-radioactive Tris-ATP, Sigma, to a specific activity of 1 to 6×10^7 c.p.m. nmol⁻¹) with or without cyclic AMP. The reaction mixture in a final volume of 0.06 ml contained 0.66 mg protein per ml, 50 mM sodium acetate buffer (pH 6.5), 10 mM magnesium acetate and 3 μ M ATP. After 1 min incubation at 30 °C the reaction was stopped by the addition of sodium dodecyl sulphate (SDS) solution to a final concentration of 3% SDS containing 5 mM Tris-acetate (pH 8.0), 1 mM EDTA, 2% 2-mercaptoethanol, 6% sucrose and 0.01% bromophenol blue which served as tracking dye. The solubilised reaction products were heated in boiling water for 2 min. Aliquots (20 μ g protein) were applied to 7–14% linear gradients of polyacrylamide gels that had been polymerised in the Hoffer (California) SE-500-1.5 slab gel electrophoresis unit, and electrophoresed using SDS-discontinuous buffer system and the Ortex 4100 pulsed constant power supply. The gels were subsequently stained with Coomassie-brilliant blue-R, dried in the Hoffer SE-540 gel drier and autoradiographed by placing them in close contact with Kodak-X-Omat X-ray film. Under the electrophoretic conditions used, inorganic phosphate, ATP and lipid bound phosphate migrated more rapidly than the phosphoproteins and did not interfere with their separation. Molecular weight estimations were obtained by electrophoresis of marker proteins on the same gel slabs. The specific activity of γ -³²P-ATP was 3.6×10^7 c.p.m. nmol⁻¹ and autoradiographic exposure time was 70 h. (–) and (+) respectively refer to the addition of water or 5 μ M cyclic AMP with the initiating labelled ATP. (–) and (+) also refer to the inclusion of 2 μ g RO20-1724 per 60 μ l (+) or solvent (–) during a 20-min preincubation period.

Table 1 Endogenous phosphorylation of five protein components in the cytosols of control and 'differentiated' neuroblastoma cells

	³² P-phosphate incorporated (arbitrary units)									
	Band 97		Band 88		Band 72		Band 59		Band 55	
	H ₂ O	Cyclic AMP	H ₂ O	Cyclic AMP	H ₂ O	Cyclic AMP	H ₂ O	Cyclic AMP	H ₂ O	Cyclic AMP
Control cells	105 ±5.2	104 ±2.4	46 ±0.5	59 ±0.7	45 ±0.5	58 ±0.9	37 ±0.7	68 ±1.0	44 ±1.9	61 ±1.8
'Differentiated' cells	60 ±2.9	63 ±2.4	58 ±4.9	69 ±1.9	63 ±2.4	77 ±1.4	66 ±3.1	137 ±2.9	61 ±3.2	83 ±3.4

RO20-1724 was used to induce differentiation^{11,12}. Cytosol (100,000g supernatant) was prepared and used in the assay of endogenous phosphorylation as described in legend to Fig. 1. Incorporation of ³²P-phosphate was determined by analysis of densitometric scans of the autoradiograms (Fig. 2) according to the recommendations of Ueda *et al.*¹⁷. The results were expressed in arbitrary density units. The results of quantitation by this method were found to be highly correlated ($+0.87$) with quantitation achieved by scintillation counting of corresponding gel slices²⁰. Each value in this table represents the mean $\pm$ s.e.m. obtained from three separate preparations which were assayed using γ -³²P-ATP with a specific activity of 3.6×10^7 c.p.m. nmol⁻¹ in the presence or absence of 5 μ M cyclic AMP. Two additional preparations were assayed with ATP of higher specific activity and gave essentially the same results. The latter were not included in the table in order to avoid undesirable normalisation of the data.

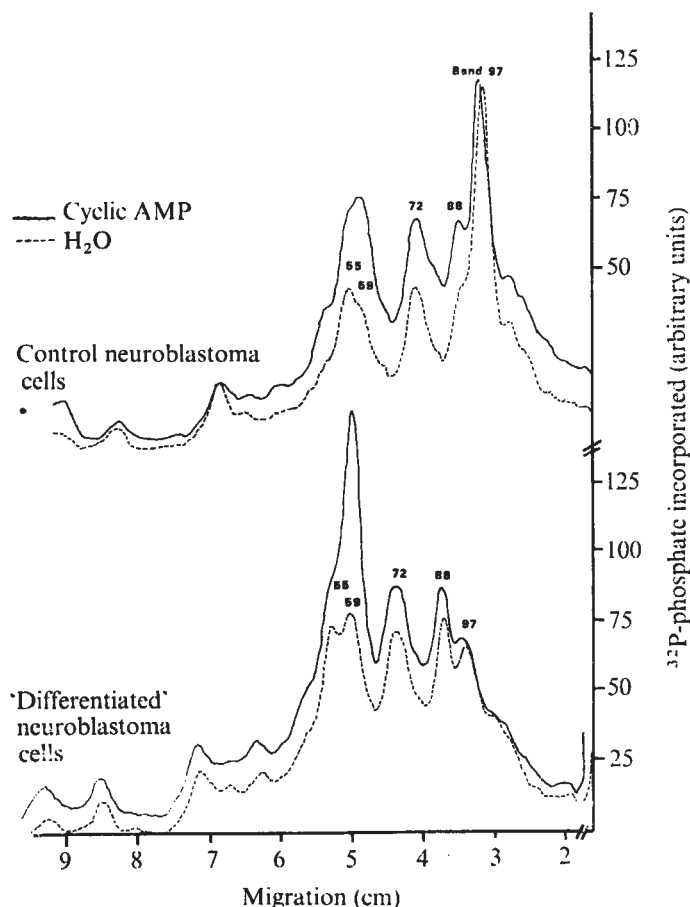


Fig. 2 Densitometric tracings of autoradiograms obtained from polyacrylamide gels after the electrophoretic separation of ^{32}P -phosphate labelled phosphoproteins of control and 'differentiated' cell cytosol. Assays of endogenous phosphorylation in the absence (---) or presence (—) of $5\ \mu\text{M}$ cyclic AMP were carried out as described in the legend to Fig. 1. The autoradiograms were then scanned at $649\ \text{nm}$ using a Beckman Acta CV spectrophotometer equipped with a linear transport system. Note that the pattern obtained with samples from control cells is characterised by predominance of band 97, while the 'differentiated' cells are characterised by the predominance of band 59.

from control and 'differentiated' cells were preincubated for 20 min at 30°C in the presence of RO20-1724 ($2\ \mu\text{g}$ per $60\ \mu\text{l}$) and the phosphorylative assay was carried out in the presence of the drug ($1\ \mu\text{g}$ per $60\ \mu\text{l}$). This treatment did not alter the phosphorylation patterns (Fig. 1). Thus, the *in vitro* assays of endogenous phosphorylation reflect phosphorylative modifications which had been pharmacologically induced in the intact cells.

The changes in cyclic AMP-dependent and cyclic AMP-independent phosphorylation found in the cytosol of 'differentiated' relative to control cells correlated well with the increase in binding of cyclic AMP by proteins localised in the cytosol of 'differentiated' cells. However, the question of whether the cyclic AMP-binding proteins identified in the cytosol of neuroblastoma cells^{11,12} only constitute regulatory subunits of cyclic AMP-dependent protein kinases, or whether some of them can directly affect inductive processes during differentiation, remains to be answered. Preliminary observations in this laboratory have indicated that the phosphorylative alterations that occur in the cytosol were accompanied by an increase in the endogenous phosphorylation of two specific proteins in the crude nuclear fraction. Thus, the observations reported here may be relevant to mechanisms that operate in inductive processes¹⁸.

The changes in phosphorylative activity observed in 'differentiated' cells raises several questions. Do these

changes occur after the division rate has slowed down or do they act as biological signals to turn off cell division? Do the phosphorylative changes precede the induction and/or the increase in various differentiated functions¹⁻¹⁰ or do they follow the expression of these functions? Do these phosphorylative changes depend upon the elevation of intracellular levels of cyclic AMP or can other agents, which are known to induce some differentiation¹ without changing the cyclic AMP level, produce similar phosphorylative changes? Are phosphorylative modifications involved in the differentiation processes that occur during normal brain development? It has been suggested that cyclic AMP, in some way, increases the expression of some genes and suppresses the expression of others during differentiation of neuroblastoma cells^{3,10,19}. The present findings, which demonstrate both increases and decreases in the phosphorylation of specific proteins during differentiation, lend support to these concepts. The ability of cyclic AMP to produce phosphorylative changes which persist for a long duration was previously demonstrated²⁰. The present study indicates that changes of this nature may have a role in neuronal differentiation.

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Regulation of cyclic GMP in cerebellum by a striatal dopaminergic mechanism

THE cerebellum has an important function in the coordination of movement and in the regulation of posture. Neurophysiological studies have established that Purkinje cell activity is stimulated by mossy and climbing fibres, which are two distinct afferent excitatory pathways to the cerebellum¹. Biochemically, an increase or a decrease in the activity of mossy and climbing fibres together with either

an activation or inhibition of gamma aminobutyric acid (GABA) synapses of Golgi, basket and Purkinje cells results in changes of the cyclic 3',5'-guanosine monophosphate (cyclic GMP) content of the cerebellar cortex²⁻⁵. Therefore, by measuring cyclic GMP in the cerebellar cortex one might infer whether drugs which influence the planning of motor activity and modify operant behaviour also change the activity of the excitatory cerebellar afferents or the function of the inhibitory intracerebellar neurones which presumably employs GABA as transmitter.

We recently reported⁶ that an antagonist (haloperidol) and an agonist (apomorphine) of dopamine receptors^{7,8} respectively decrease or increase the cyclic GMP content of cerebellar cortex. These changes in cyclic GMP occur without parallel modification of cerebellar cyclic 3',5'-adenosine monophosphate (cyclic AMP) content⁶. It is currently believed that the cerebellum is devoid of dopamine receptors⁹ and that the dopamine present in this structure functions as a precursor of noradrenaline¹⁰. Since neither cyclic GMP nor cyclic AMP content change as a result of *in vitro* application of dopamine to cerebellar slices¹¹ or addition of apomorphine or haloperidol to cerebellar homogenates⁶, it seems unlikely that these nucleotides function in cerebellum as second messengers of dopamine receptor activation. Hence we have inferred that haloperidol and apomorphine change the cerebellar cyclic GMP content when given parenterally by interacting with dopamine receptors located in other brain areas. An area of the brain which contains a large functional pool of dopamine receptors is the basal ganglia. Changes of cerebellar cyclic GMP could then occur as a consequence of stimulation or blockade of dopamine receptors located in basal ganglia. We have obtained preliminary evidence for such a hypothesis by comparing the changes in cerebellar cyclic GMP content elicited by intrastriatal, intracerebellar and intraperitoneal injection of different neuroleptics and apomorphine.

As shown in Fig. 1, the intraperitoneal injection of haloperidol, chlorpromazine and (+)-butaclamol, three drugs that presumably block dopamine receptor function^{7,8}, produced a dose-related decrease of the cyclic GMP content of cerebellar cortex; a 30% decrease was obtained with as little as 0.1 μmol per kg of haloperidol, 0.2 μmol of chlor-

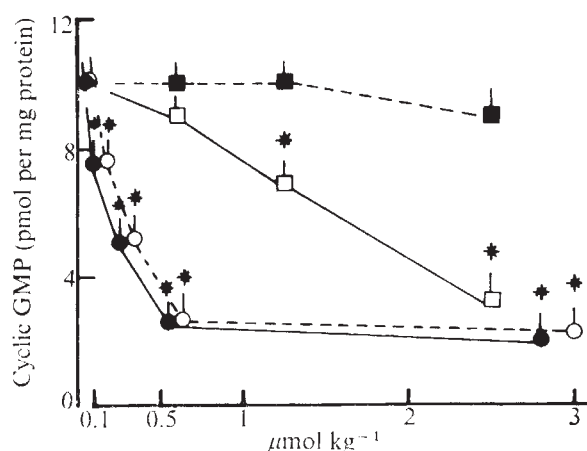


Fig. 1 Decrease in the cyclic GMP content of cerebellar cortex after various doses of haloperidol (●), chlorpromazine (○), (+)-butaclamol (□) and (-)-butaclamol (■). Haloperidol, chlorpromazine and butaclamol were injected i.p. 30 min before killing the rats. Male Spague-Dawley rats of 130–150 g were killed with a microwave radiation beam focused on the skull¹⁶. The cyclic GMP content of punches (0.8–1 mg protein) of cerebellar cortex was extracted, purified and assayed as previously reported¹⁶. Each point is the mean $\pm$ s.e.m. of six experiments. * $P < 0.01$ when compared with animals treated with solvent.

promazine and 1 μmol of (+)-butaclamol. The figure also shows that (-)-butaclamol, which fails to block dopamine receptors⁸, also failed to decrease the cerebellar content of cyclic GMP. As is evident from the data shown in Table 1, haloperidol and (+)-butaclamol decreased the content of cyclic GMP in cerebellar cortex also when they were directly infused into the striatum.

Table 1 Effect of intrastriatal and intracerebellar injection of haloperidol and (+) or (-)-butaclamol on cerebellar cortex cyclic GMP content

Drug	μg	Cerebellar cortex cyclic GMP (pmol per mg protein)	
		Intrastriatal injection	Intracerebellar injection
Solvent		10.4 $\pm$ 4.5	9.8 $\pm$ 2.0
Haloperidol	6	4.9 $\pm$ 2.3*	10.2 $\pm$ 2.6
(+)-Butaclamol	10	5.1 $\pm$ 1.8*	10.6 $\pm$ 1.6
(-)-Butaclamol	10	9.5 $\pm$ 2.1	9.6 $\pm$ 2.4

Drugs were injected either directly in striatum (AP=7.6; L=2.8; DV=+1.5)¹⁷ or in the cerebellar hemisphere (AP=3.4; L=3; DV=-1)¹⁷ through chronically implanted cannulae. The intracerebellar injections were made in a volume of 1 μl which was injected at a rate of 1 μl per 2 min. The animals were killed 30 min later.

* $P < 0.05$ when compared to animals treated with solvent. Each point is the mean $\pm$ s.e. of four experiments. Rats were killed 30 min after drug injection.

AP=Anterior-Posterior; L=Lateral; DV=Dorsoventral.

A 50% reduction of cerebellar cortex cyclic GMP was obtained by intrastriatal injection of 6 μg of haloperidol or 10 μg of (+)-butaclamol. In contrast, both drugs failed to change cerebellar cyclic GMP content when directly applied to the cerebellum. Moreover, the inactive isomer (-)-butaclamol did not change cerebellar cyclic GMP content after either intrastriatal or intracerebellar injection. Thus the results support the idea that the functional state of striatal dopamine receptors may be responsible for changes of cerebellar cyclic GMP.

In order to determine whether the decrease of cerebellar cyclic GMP content elicited by intraperitoneal injection of antipsychotics was mediated by a blockade of striatal dopamine receptors or by blockade of dopamine receptors located in other brain areas we studied the action of apomorphine injected intrastrially on the cyclic GMP content of cerebellar cortex. As shown in Table 2, apomorphine (10 μg) injected in both striata increased cerebellar cyclic GMP in control animals and also completely reverted the decrease of cerebellar cyclic GMP in animals pretreated intraperitoneally with haloperidol. Hence, our results demonstrate that the content of cerebellar cyclic GMP can be influenced by activating dopamine receptors in basal ganglia.

The interaction between basal ganglia and cerebellum may occur at various levels. Striatum and cerebellum are

Table 2 Effect of an intrastriatal injection of apomorphine on cerebellar cortex cyclic GMP of rats pretreated with haloperidol

Drug	Cerebellar cortex cyclic GMP (pmol per mg protein)	
	Solvent (1 μl)	Apomorphine (10 μg μl^{-1})
Solvent 5 ml kg^{-1} i.p.	9.8 $\pm$ 0.36	15.2 $\pm$ 0.21*
Haloperidol 0.14 μmol kg^{-1} i.p.	7.5 $\pm$ 0.18*	11.1 $\pm$ 0.41†

Apomorphine was injected in both striata through chronically implanted cannulae. Rats were killed 6 and 31 min after apomorphine and haloperidol respectively. Each point is the mean $\pm$ s.e. of five experiments.

* $P < 0.001$ when compared with animals treated with solvent.

† $P < 0.02$ when compared with animals treated with 0.14 mol kg^{-1} of haloperidol.

both involved in the coordination and regulation of motor activity and operant behaviour. Both cerebellum and basal ganglia receive a substantial somatosensory input from the cortex, they filter this information and project their modulatory influence back to the cortex through a multi-synaptic pathway by way of the ventrolateral thalamic nuclei^{12,13}. It has been proposed that cerebellum and basal ganglia act in parallel to set up patterns of thalamus cortical output which is necessary for the appropriate activation of cortico-spinal motor neurones¹². Moreover, neurophysiological data indicate a direct link between basal ganglia and cerebellum suggesting that changes in the activity of striatal neurones can produce parallel changes in the neurones of the cerebellar cortex^{14,15}. It is therefore tempting to suggest that the measurement of cerebellar cyclic GMP may be a sensitive biochemical index of the impairment of striatal function elicited by antipsychotics. These results may also be interpreted as preliminary evidence for a cerebellar role in some of the actions of neuroleptic drugs.

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Excitatory action of TRH on spinal motoneurones

THE recent isolation and characterisation of a number of peptide hypothalamic-releasing (and release-inhibiting) hormones led to an unexpected bonus. Many of these peptides have been found to be widely distributed in brain in areas remote from established hypothalamic neuroendocrine systems. For instance, two thirds of the brain thyrotropin-releasing hormone (TRH) is located outside the hypothalamus^{1,2}. This finding suggests that TRH might have some neuroregulatory role in the brain besides its role as a releasing hormone. The marked behavioural effects of central origin produced by TRH support this notion³⁻⁷. In particular, the finding that TRH can be localised by immunohistochemical techniques to nerve fibres in a variety of brain regions⁸, that it depresses neuronal activity when applied by microiontophoresis⁹⁻¹¹ and that it has stereoselective, high-affinity binding sites in brain¹² suggests that TRH may be a neurotransmitter. There is little evidence, however, on the transmembrane effects and mechanism of action of TRH or other peptides on central neurones.

Spinal motoneurones in the rat receive a moderate density of nerve fibres which are immunoreactive for TRH⁸. This finding provides an opportunity to analyse with intracellular recording the effects of TRH on the membrane properties of central neurones. I show here that TRH has a purely excitatory action with a potency similar to that of glutamate when applied to frog motoneurones. The action of TRH also resembles that of glutamate in that it is often accompanied by an increase in conductance of the membrane. It differs, however, from that of glutamate in that the maximum depolarisation rarely surpasses threshold suggesting that TRH provides a background facilitatory action on motoneurones. The structural requirements for this depolarising action parallel those for the release of thyrotropin¹³. Although immunohistochemical localisation studies have not been performed in frogs, particularly high concentrations of TRH are found in extrahypothalamic tissue¹.

Rana pipiens were chilled on ice and the spinal cord removed, hemisected and placed in a sucrose gap chamber. The activity of motoneurones was recorded by placing a filament of the ventral root across the sucrose gap and the potential difference across the gap was monitored with two calomel electrodes. An upward deflection indicated that the bath electrode was negative relative to the root electrode, signifying a depolarisation of the motoneurones. The cord was perfused with an oxygenated (100%) Ringer solution of the following composition: 114 mM NaCl, 2 mM CaCl₂, 2 mM KCl and 10 mM Tris buffer, pH 7.3. The temperature was maintained at 18 °C. For intracellular recording glass microelectrodes with tip resistance between 15 and 20 MΩ were selected. Motoneurones were identified by antidromic invasion and only those cells with stable membrane potentials of -65 mV or more were selected for study. The responsiveness of the motoneurone pool was continuously monitored with sucrose gap recording during the intracellular studies. Responses were recorded on a chart recorder and also photographed from the oscilloscope screen. TRH was purchased from Beckman Instruments, Peninsula Laboratories, and Calbiochem Laboratory. All samples gave similar results.

Sucrose gap recording revealed the TRH had a depolarising potency comparable to that of glutamate (Fig. 1a). Its action differed from that of glutamate in a number of ways. It was of considerably longer duration (Fig. 1a), considerably more variable from preparation to preparation, often showed tachyphylaxis, and the peak of the dose response curve was only 3.7 ± 1.4 mV (s.d.), whereas high concentrations of glutamate resulted in depolarisations of 10 to 20 mV. It is possible that the rapid breakdown of the TRH molecule contributes to the small size of the response, although the addition of a peptidase inhibitor, bacitracin⁴, (5×10^{-5} M) (Calbiochem) or lowering the temperature did not increase the size of the response. Blockage of synaptic transmission either with magnesium ions (10 mM) or tetrodotoxin (5×10^{-6} M) reduced the size of the TRH response by 10-40%, suggesting that there are both direct and indirect components to the depolarisations. Equimolar concentrations of pyroglutamate, histidine, or prolinamide, the amino acid constituents of the TRH molecule, either had no effect or an inhibitory action, indicating that the depolarisation cannot be explained by the activation of amino acid receptors by one of these amino acids following breakdown of TRH to its constituent amino acids. Furthermore, the 1-methyl-histidine-TRH analogue was devoid of activity, while the 3-methyl-histidine-TRH analogue was approximately 10 times more potent than TRH.

Intracellular recording from motoneurones ($n=43$) confirmed that TRH had a depolarising action and that maximum depolarising concentrations resulted in a low level depolarisation ($5.6 \text{ mV} \pm 3.23 \text{ s.d.}$, $n=43$) which never

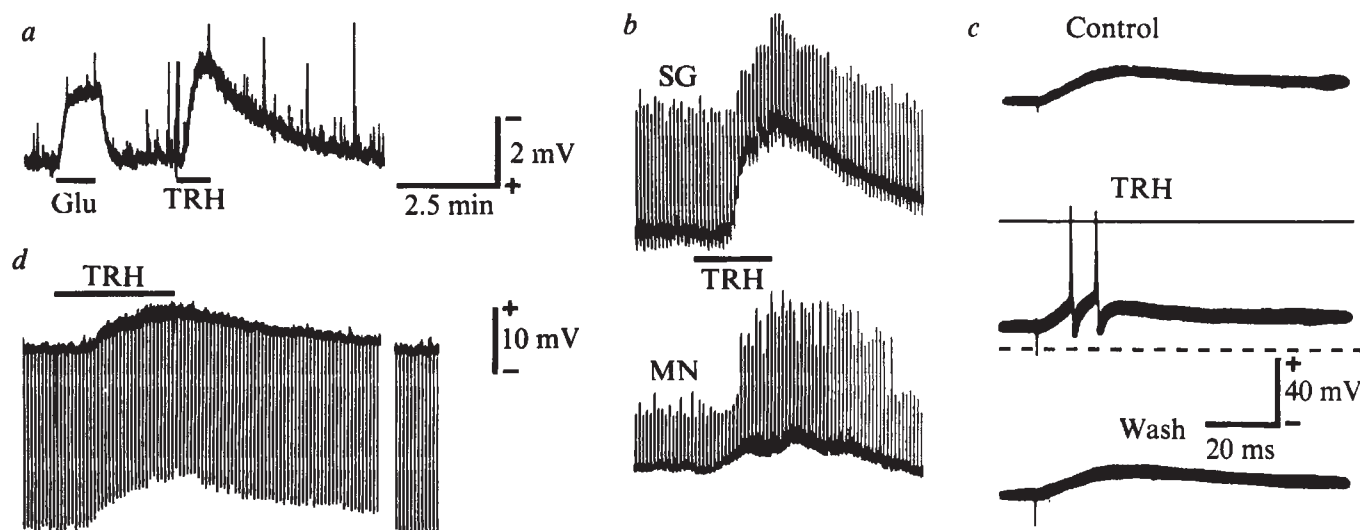


Fig. 1 Depolarising action of TRH on frog motoneurons. *a*, depolarisation of motoneurons to L-glutamate (10^{-4} M) and TRH (10^{-4} M) recorded with sucrose gap from a ventral root. The rapid depolarising deflections result from spontaneous synaptic input to the motoneurons. In *b*, subthreshold e.p.s.p.s which appear as upward deflections reach threshold during TRH depolarisation (5×10^{-4} M). Top trace (SG) is sucrose gap pen record from ventral root and bottom record (MN) is the intracellular response simultaneously recorded from a motoneurone (resting potential, -77 mV). Dorsal root was stimulated every 5.5 s. Spikes during depolarisation in lower record are attenuated in the pen record. *c*, Film records showing e.p.s.p.s and spikes from another motoneurone (resting potential, -75 mV). In *d*, constant current hyperpolarising pulses were applied through the microelectrode every 5.5 s which appear as downward deflections (resting membrane potential, -85 mV). The membrane resistance fell from 3.1 M Ω to 2.8 M Ω during TRH. The gap in the record is 5 min. The time calibration in *a* also applies to the other chart records. The voltage calibration in *a* (negativity up) also applies to the sucrose gap record in *b*. The voltage calibration in *d* (negativity down) also applies to the intracellular record in *b*.

reached firing threshold in uninjured cells. This depolarisation did, however, have an excitatory action, raising subthreshold excitatory postsynaptic potentials (e.p.s.p.s) and depolarising current pulses above threshold. This is shown for e.p.s.p.s in Fig. 1*b* (bottom record) and *c*, from two different motoneurons. To analyse the mechanism underlying the depolarisation, membrane resistance was monitored by passing constant current hyperpolarising pulses. In 11 out of 21 motoneurons examined a small decrease in resistance occurred during the depolarisation (Fig. 1*d*). The inability to detect a resistance change in some cells is probably due to the small size of the depolarisation and to limited current carrying capacity of the microelectrodes. The resistance change seen with TRH was of a similar magnitude to that produced by an equivalent depolarisation by glutamate, suggesting that TRH increases conductance to sodium ions.

In summary, the present results indicate that TRH produces in frog motoneurons a long duration depolarisation of small magnitude which is accompanied by an increase in membrane conductance and excitability. Very similar results have been observed with the peptide substance P (refs 15, 16 and unpublished observations). Such actions suggest that TRH and substance P act in a background manner to facilitate transmission through more powerful synaptic pathways subserving reflex activity. The excitatory action of TRH on motoneurons is in contrast to its inhibitory action on supraspinal neurones⁸⁻¹¹ raising the possibility that it may act as an excitatory transmitter on one cell type and as an inhibitory transmitter on another. In this regard the excitatory action described in the present report is perhaps more consistent with the behavioural effects of TRH which are generally of a stimulant nature³⁻⁷, and with its ability to release thyrotropin from the anterior pituitary, the latter being associated with an excitation of anterior pituitary cells¹⁷. Interestingly the structural requirements for the behavioural effects¹⁸, releasing activity¹⁹, and membrane excitatory actions reported here are all quite similar. The direct neuronal effects of TRH strengthen the concept that TRH may play a neurotransmitter role in addition to and possibly unrelated to its well established

neuroendocrine role, although similar receptors are involved in both functions.

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Saturation of a retinal cone mechanism

In discussions of the several differences between the rods and the cones of the human retina it is usually stated that rods saturate and cones do not. When measurements are made of the intensity (ΔI) of an incremental flash that can just be detected on a steady background field of intensity I , the cones normally continue to obey Weber's law ($\Delta I/I = \text{constant}$) at very high intensities when nearly all their pigment has been bleached away; but when only a very small fraction of rhodopsin, the rod pigment, has been bleached the incremental threshold for

rods begins to rise more rapidly than is predicted by Weber's law. This saturation of the rod signal can be observed when, by careful choice of wavelengths and by exploitation of the directional selectivity of the cones, the rod response is followed to high intensities in normal observers¹; or when measurements are made on rod monochromats, whose retinæ completely, or almost completely, lack cone receptors²⁻⁴. Alpern, Rushton and Torii⁵ have suggested that it is bleaching that prevents saturation in the cones, for the receptor signal depends on the number of quanta actually absorbed and the latter quantity will tend to a limit as more and more pigment is bleached by steady background fields of increasing intensity. Cone mechanisms do saturate when intense, brief flashes are delivered to the unbleached retina⁶⁻⁸. We show here that the blue-sensitive receptors, which are normally thought to be cones, can be saturated by steady fields. In this respect they resemble the rods rather than the red- or green-sensitive cones.

Since the green cone mechanism is almost as sensitive to blue light as is the blue mechanism⁷, it was necessary to choose experimental conditions carefully in order to follow the isolated response of the blue mechanism to high intensities. The test flash was violet (435 nm); and was large (1° of visual angle) and long (200 ms) in order to take advantage of the spatial and temporal integrative properties of the blue mechanism⁸⁻⁹. It was delivered to the fovea, fixation being guided by an array of four small fixation lights arranged in a diamond. A bright yellow (575-nm) 'auxiliary field'¹⁰, which subtended 6.5°, served to maintain the light adaptation of the long- and middle-wavelength mechanisms; it produced a retinal illuminance of 5.48 log troland (equivalent to 0.17 log erg s⁻¹ degree⁻²) and remained present throughout the experiment. The primary adapting field (445 nm) was of variable intensity and was congruent with the auxiliary field. The arrangement of the

stimuli is shown in Fig. 1d. The experiment was under computer control and a random double-staircase procedure¹¹ was used to measure the threshold for detecting the violet test flash at each of an increasing series of intensities of the 445-nm adapting field. Four minutes of light adaptation preceded measurements at each new level of the 445-nm field. Other details of procedure, apparatus and calibration were as described elsewhere¹².

Results are shown in Fig. 1a and b. The broken line has a slope of 1 and represents Weber's law. Clear evidence of the onset of saturation is seen in the rapidly rising increment-threshold function. Blue-violet fields that cause saturation are not very bright: the increment-threshold function exceeds a slope of 1 when the 445-nm field has a value of ~100 trolands, although, of course, the photopic troland is not an appropriate measure of light absorbed by the short-wavelength receptors.

At the highest field intensities the function suddenly flattens, and we suggest that here the green cones have taken over the detection of the violet flashes. To test the latter proposal directly we measured the spectral sensitivity of the eye when it was adapted to the most intense 445-nm field, the auxiliary field still being present: different test wavelengths were used in random order and measurements were always made between 4 and 10 min after the onset of adaptation, during which period the threshold is known to be stable for the observer used (J.D.M.). Measurements were made at two wavelengths in each session and at least 3 h elapsed between sessions. The results (Fig. 1c) are well fitted by π_4 , Stiles's green-sensitive cone mechanism⁷. They could in principle be fitted by the red mechanism, π_3 , but definitely not by π_2 , the blue mechanism (which is plotted in Fig. 2c).

The reader will ask why saturation of the blue receptors has not previously been recorded, either by Stiles or by the many

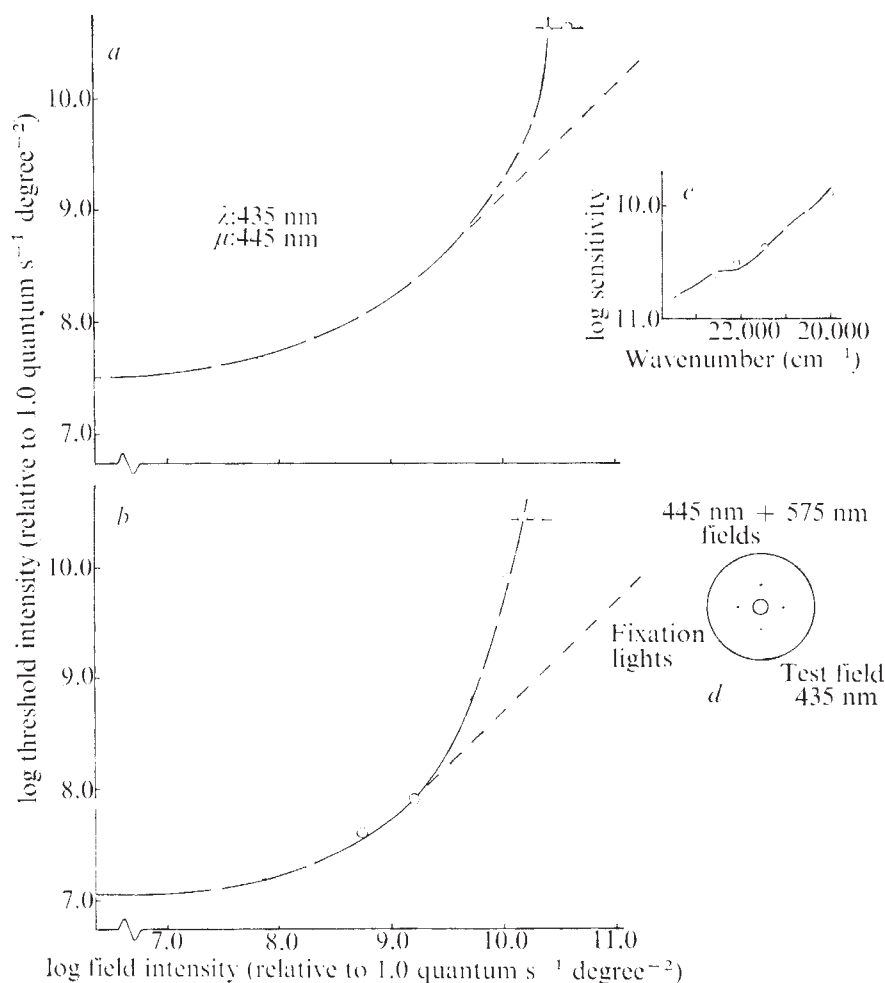


Fig. 1 a, b, Incremental threshold (ΔI) for the 435-nm test flash as a function of the 445-nm primary adapting field. The left-most point represents the threshold on the yellow auxiliary field alone. Observers: J.D.M. (a), P.G.P. (b). c, Spectral sensitivity of the eye after 4 min adaptation to the most intense 445-nm field (10.57 log quanta s⁻¹ degree⁻²). The ordinate is log sensitivity, that is, log reciprocal threshold (relative to 1.0 quantum s⁻¹ degree⁻²). The solid line represents the spectral sensitivity of Stiles's green mechanism, π_4 , and has been arbitrarily displaced vertically. d, Arrangement of the stimuli.

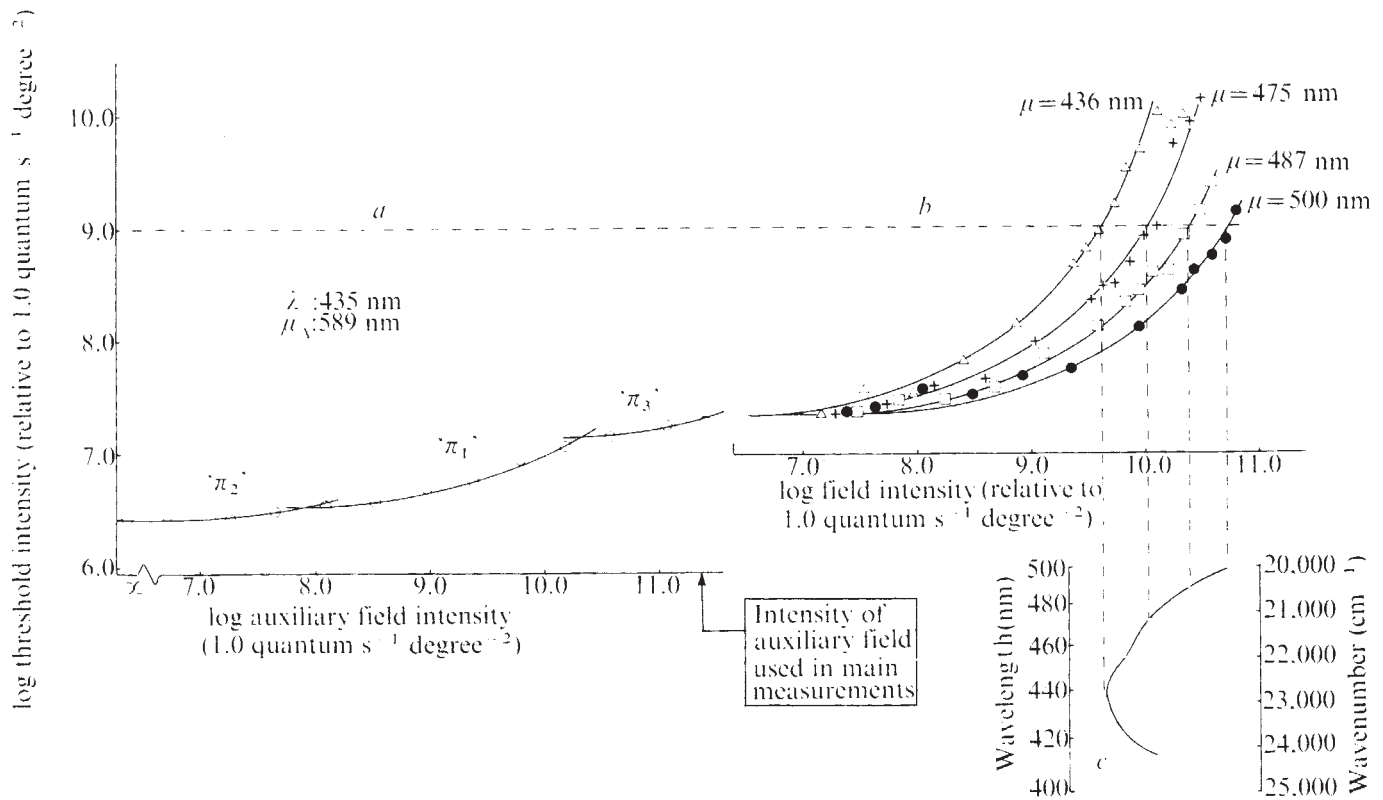


Fig. 2 *a*, Incremental threshold for a 435-nm test flash as a function of the intensity of the orange field alone. The curves fitted to the data points in each case correspond to Stiles's function $\zeta(x)$, taken from Table 7.5, ref. 7; and the relative position on the ordinate of the π_1 and π_3 branches is derived from the tabulated sensitivities of these mechanisms (Table 7.6, ref. 7). The reader should place less weight on the π_2 branch, which has been arbitrarily placed. Observer: J.D.M. *b*, Incremental threshold as a function of the intensity of the main adapting field. The parameter is the wavelength of the main adapting field (μ). The auxiliary field was present throughout. The solid curve is empirical and has been displaced laterally to give the best fit to each set of data. Note that quanta absorbed from the auxiliary field are not represented by the abscissa of this plot. (The theory of the auxiliary field is discussed by Stiles in ref. 10). *c*, Field intensities that produce criterion saturation plotted against wave number. The solid line represents the field sensitivity of Stiles's high-intensity blue mechanism, π_3 .

others who have measured increment thresholds using his two-colour procedure. A simple explanation is that many experimenters have not had available the necessary intensities of short wavelength light. But since Stiles is usually cited in support of the claim that cones are never saturated by steady fields, it is interesting and reassuring to note that Fig. 11 of the paper¹⁰ published by Stiles in 1953, which represents data collected in conditions similar to those used here, does show an increment threshold function with a final slope that is greater than 1. Stiles used a 555-nm auxiliary field of $-2.15 \log \text{erg s}^{-1} \text{ degree}^{-2}$, which was less intense than that of the present experiment: thus the intrusion of π_1 would occur earlier and would mask the most steeply rising part of the function of Fig. 1*a* and *b*. Norren and Padmos¹⁹ have presented electroretinographic evidence that the dynamic range of the blue mechanism is limited.

What causes Weber's law to fail for the blue receptors? There is good evidence that at lower intensities the blue mechanism is subject to inhibition from a long-wavelength mechanism and that Stiles's three blue mechanisms, π_1 , π_2 , and π_3 , correspond to the same receptors in different states of inhibition (for discussion see refs 12 and 13). Does the saturation seen in Fig. 1 arise directly from quanta absorbed by the short-wavelength receptors or is the blue mechanism being inhibited by a mechanism with a different spectral sensitivity? If the former is the case, we should find the lights of different wavelength that produce a given degree of saturation are lights that from other evidence would be thought to produce equal absorption in the blue receptors; but if the alternative hypothesis were correct, then the results of varying the wavelength of the adapting field ought to reflect the spectral sensitivity of the putative inhibitor. These expectations were the basis for a second experiment, in which the wavelength of the primary field was varied.

The conditions for the second experiment were similar to those of the first, but the wavelength of the auxiliary field (μ_A) was lengthened from 575 to 589 nm, in order better to reveal Stiles's π_3 mechanism, and its brightness was reduced to 5.2 log troland ($-0.03 \log \text{erg s}^{-1} \text{ degree}^{-2}$). The left-hand panel of Fig. 2 shows the effect of the orange auxiliary field alone on detection of the violet test flash. The three shallow branches correspond to Stiles's mechanisms π_1 , π_2 , and π_3 . The final level of the orange field (marked with an arrow) was that used as the auxiliary field during the main measurements and it is seen to raise the threshold by about one log₁₀ unit.

Primary adapting fields of varying wavelengths were then added to the orange auxiliary field, a single experimental session being devoted to each wavelength. Results for four adapting wavelengths are shown in Fig. 2*c*; the results for other wavelengths are not shown to avoid congestion on the plot, but they are very similar in form.

Each set of data can be fitted well by a curve of fixed form that obeys Stiles's displacement rule¹⁴, being displaced along the abscissa without vertical shift or other distortion. Figure 2 shows graphically how from these data can be derived the spectral sensitivity of the mechanism underlying saturation. We take as an arbitrary criterion that field intensity at which the slope of the increment-threshold function is 2. (Since the curve is of fixed shape, any criterion will give the same answer.) For each adapting wavelength this value is projected downwards and plotted against wavelength together with the corresponding points for sets of data not shown. The spectral sensitivity function that results (open triangles, Fig. 2*c*) is extremely well fitted by Stiles's curve for π_3 , which is taken from ref. 7 (Table 7.6) and which can on several grounds be regarded as a good estimate of the spectral sensitivity of the blue receptors. That the fit is good must follow from the fact that the increment threshold

functions of Fig. 2b have a fixed shape, for Stiles's measurements of the field sensitivity of π_3 were made in similar conditions but with a lower criterion.

Basic to this derivation is the assumption that Rushton¹⁵ has called the principle of univariance: the output of a receptor depends only on the number, and not on the wavelength, of the quanta caught. As we vary wavelength, all that will change is the proportion of incident quanta that are absorbed. If the data of Fig. 2b could be plotted in terms of quanta actually absorbed then all the curves would coincide; but the abscissa is in fact the number of quanta delivered to the cornea and thus each curve is displaced laterally by a distance equal to the log of the ratio of quanta incident to quanta absorbed. It is these displacements that give us the spectral sensitivity of that which causes the saturation.

We conclude that the blue mechanism saturates when the blue-sensitive receptors are themselves absorbing a fixed number of quanta from a field. The present experiment offers no evidence for inhibition.

The saturation of the blue receptors at relatively low intensities may account for several long-standing problems of colour vision. It may, for example, explain why violet lights show a Bezold-Brücke hue shift towards longer wavelengths, coming to appear pale blue as their intensity is increased¹⁶⁻¹⁷.

We have some evidence that if adaptation is maintained at saturating levels for more than 15 min, a recovery of sensitivity occurs: this further anomaly, which recalls physiological findings for rods in the skate¹⁸, is under investigation.

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ATP-dependent calcium storage in presynaptic nerve terminals

CALCIUM ions, which enter presynaptic nerve terminals during depolarisation, trigger transmitter release from the terminals of both peripheral¹ and central² neurones. The subsequent disposition of the entering Ca is not entirely settled. Although this Ca must eventually be extruded in order for the neurones to return to the steady state, some of it may be temporarily retained and sequestered in intraterminal organelles. We now present evidence for an intraterminal, ATP-dependent Ca storage system which may help to regulate internal ionised Ca concentrations. This latent Ca sequestering system has been revealed by lysis of pinched-off presynaptic nerve terminals ("synaptosomes") in hypotonic media, followed by measurements of

ATP-dependent Ca uptake from media containing mitochondrial inhibitors. These observations provide an alternative to the suggestion^{3,4} that intraterminal mitochondria may be the primary Ca storage sites.

Synaptosomes were prepared from homogenates of rat brain cerebral cortex by the procedure of Hajós⁵. The synaptosomes (in 30-40 ml of 0.8 M sucrose), were diluted⁶ to about 200 ml with an ice-cold buffered physiological salt solution (145 mM NaCl, 5 mM KCl, 1.3 mM MgCl₂, 2.4 mM NaH₂PO₄ and 20 mM HEPES [N-2-hydroxyethyl piperazine-N'-2-ethanesulphonic acid] buffered to pH 7.65 at 20 °C with Tris [hydroxymethyl] aminomethane base); the protein concentration, after dilution, was 0.11-0.15 mg ml⁻¹. Aliquots of the saline-diluted suspensions were centrifuged at 15,000g for 5 min at 5 °C. The supernatant solutions were discarded, and the synaptosome pellets were resuspended and used for the ⁴⁵Ca uptake studies described below (see figure legends for details). In each experiment, several representative pellets were assayed for protein by the Lowry method⁷.

Following incubation in ⁴⁵Ca-labelled fluids, aliquots (containing 0.2-0.3 mg protein) of the suspensions were pipetted on to 2.5 cm diameter, 0.3 µm pore diameter Millipore filters (Millipore, Bedford, Massachusetts). The fluid was filtered by suction, and the filters were washed three times with 4 ml aliquots of ice-cold physiological salt solution (see above) containing 1.2 mM CaCl₂. The ⁴⁵Ca content of the material on the filters, and of samples of the ⁴⁵Ca incubation solutions, was determined by standard liquid scintillation spectrometry.

There is considerable evidence that synaptosomes reseal and exhibit functional properties similar to those of intact nerve terminals when incubated in physiological salines^{2,6}.

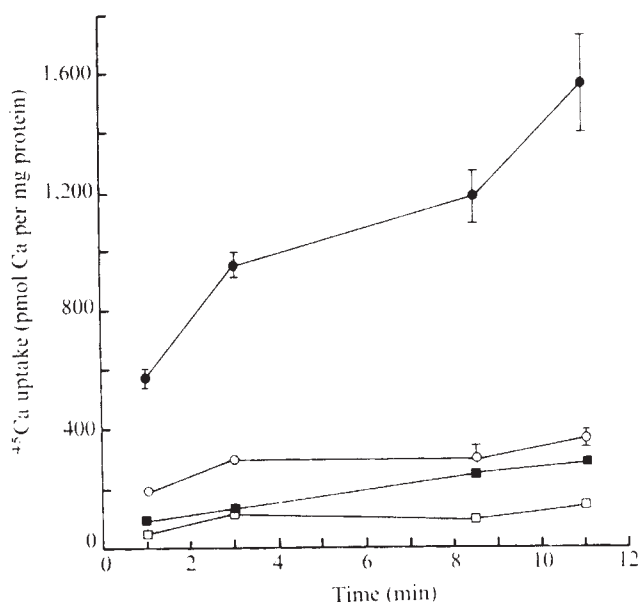


Fig. 1 Time course of ⁴⁵Ca uptake by intact and disrupted synaptosomes in the presence and absence of ATP. The "intact" synaptosomes (□, ■) were incubated in 2 ml of the standard physiological salt solution (see text) containing 10 µM CaCl₂ labelled with ⁴⁵Ca; the solution also contained 0.1 mM DNP, 0.1 mM Na azide and 0.7 µg ml⁻¹ oligomycin. Other (○, ●) "disrupted" synaptosomes (○, ●) were resuspended and incubated in 2 ml of a solution similar in composition except for the omission of all the NaCl; the sudden exposure to the markedly hypotonic media presumably induced lysis⁸. When 2 mM ATP was present (●, ■), the MgCl₂ concentration in the incubation fluid was increased to 3 mM; open symbols refer to tissue incubated in ATP-free solutions. Incubations were carried out at 30 °C; the protein concentration in the suspensions was 0.41 mg ml⁻¹. Each symbol represents the mean of three determinations; error bars are shown where the standard errors extend beyond the borders of the symbols.

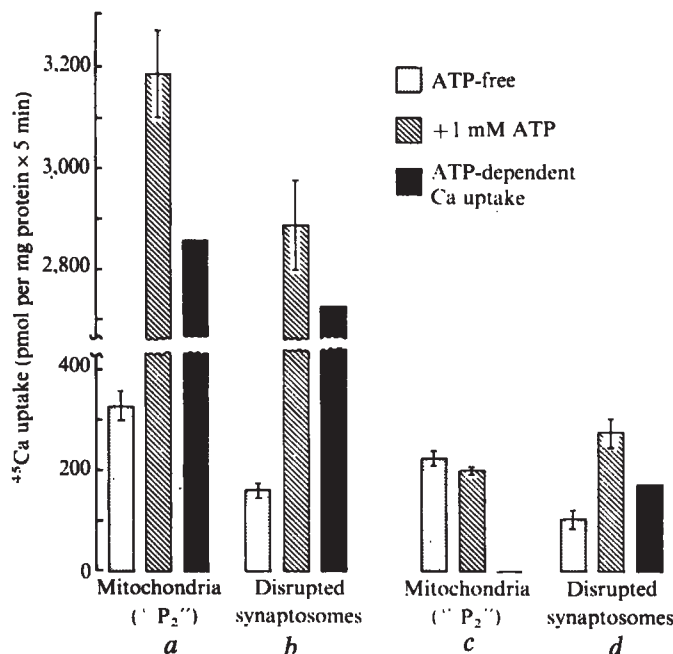


Fig. 2 Comparison of ^{45}Ca uptake by mitochondria and disrupted synaptosomes. The "crude mitochondrial pellet" ("P₂", ref. 10) was suspended in about 30 ml of ice-cold 0.32 M sucrose. Half of the suspension was used to prepare a synaptosome-enriched fraction by the method of Hajós⁹. The remainder was immediately diluted with about 100 ml of ice-cold standard physiological saline; isotonic solutions were used in order to avoid lysis of the nerve terminals present in this fraction (see Fig. 1). Aliquots (6 ml) were pipetted into centrifuge tubes and the particulate material was pelleted by centrifugation at 15,000g for 5 min at 5 °C. The supernatant solutions were discarded, and the pellets (1.39 mg protein) were suspended in 1.0 ml of physiological saline with all the NaCl replaced by KCl (*a, c*); the solution pipetted into some tubes (*a, c*) also contained DNP, NaN₃ and oligomycin (to give final concentrations of 0.1 mM, 0.1 mM and 0.7 $\mu\text{g ml}^{-1}$, respectively). After 1 min of incubation at 30 °C, 1 ml of similar K-rich saline containing 10 μM Ca labelled with ^{45}Ca was added; the solution added to some tubes (see graph) also contained ATP (final concentration 1 mM). Following a 5-min incubation at 30 °C, aliquots of the suspensions were filtered and treated as described in the text. The synaptosomes obtained by gradient centrifugation³ of the sucrose-suspended "P₂", were diluted with saline and centrifuged (see text). The pellets (0.93 mg protein) were suspended in 1 ml of "lysis solution" (standard physiological saline with all the NaCl omitted) (*b, d*); the solution used to suspend some of the pellets also contained the three mitochondrial poisons (*d*). After 1 min of incubation at 30 °C, 1 ml of buffered saline containing 290 mM KCl (and no NaCl) was added to each suspension; this solution contained 10 μM CaCl₂ labelled with ^{45}Ca and, where appropriate (see graph) ATP (final concentration 1 mM). Following a 5-min incubation at 30 °C aliquots of the suspensions were filtered and treated as described in the text. Each bar represents the mean of three determinations; error bars indicate s.e.m.

Figure 1 shows that when the external Ca concentration is about 10 μM , ATP has little effect on Ca uptake by intact synaptosomes incubated in the physiological salt solution with an osmolarity of about 320 mosM. If, however, the synaptosomes are disrupted by exposure to markedly hypotonic media¹, a new Ca uptake process appears—one which is greatly enhanced by ATP (Fig. 1). The fact that it is observed in lysed, but not in "intact", preparations suggests that the uptake mechanism is associated with intraterminal organelles. Following lysis, re-introduction of a 320 mosM solution does not abolish the ATP-stimulated Ca uptake (see Figs 2–4). This ability to withstand large changes in osmotic pressure is reminiscent of fragmented sarcoplasmic reticulum, which can be isolated in 30 mM KHCO₃ and then returned to an approximately 300 mosM solution for Ca uptake studies².

Participation of mitochondria in this ATP-stimulated Ca uptake can be ruled out, because all incubation solutions contained 0.1 mM 2,4-dinitrophenol (DNP) and 0.1 mM sodium azide (two mitochondrial uncouplers), and 0.7 $\mu\text{g ml}^{-1}$ oligomycin (an inhibitor of mitochondrial ATP-promoted Ca uptake); this combination of poisons blocked virtually all ($99 \pm 1\%$; $n=4$) of the Ca uptake in control

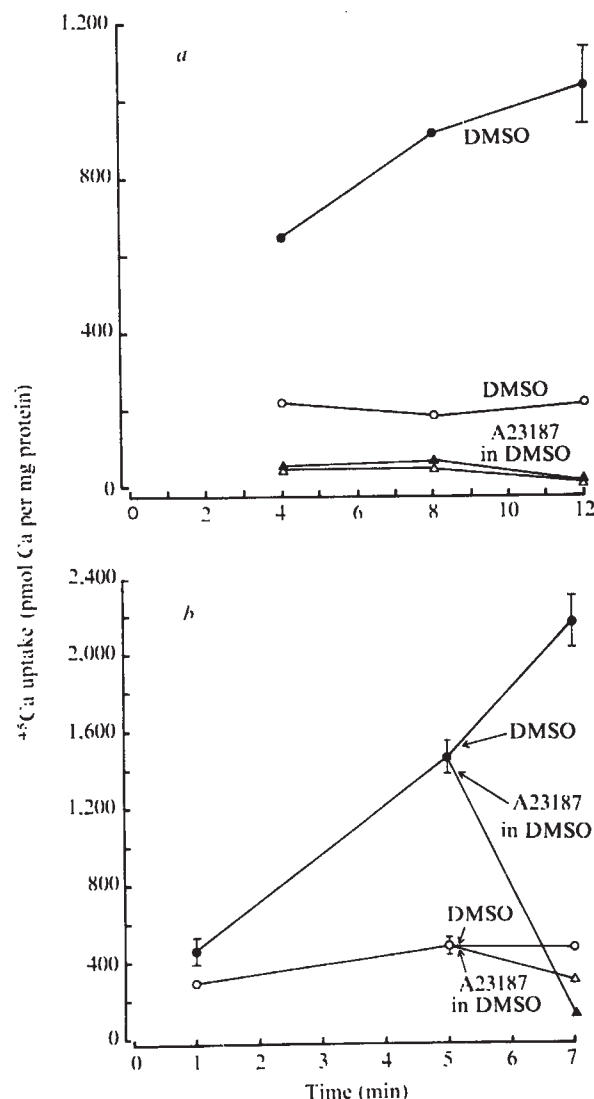


Fig. 3 Effect of ionophore, A23187, on ^{45}Ca uptake by disrupted synaptosomes. Data in *a* and *b* are from different synaptosome preparations. In both experiments, the synaptosomes were disrupted by pre-incubating them for 1 min in 1 ml of standard physiological salt solution with the NaCl omitted. Incubation fluid (1 ml) was then added to provide a (final) composition similar to that of the standard solution, but with all of the NaCl (145 mM) replaced by KCl. In addition, the incubation fluid contained sufficient ^{45}Ca -labelled CaCl₂ to give a final concentration of 10 μM in Ca; when 2 mM ATP was present (●, ▲), the MgCl₂ concentration was increased to 3 mM. All lysis and incubation fluids contained 0.1 mM DNP, 0.1 mM Na azide and 0.7 $\mu\text{g ml}^{-1}$ oligomycin. All incubations were carried out at 30 °C. Each symbol represents the mean of three determinations; error bars are shown where the standard errors ranged beyond the borders of the symbols. *a*, ^{45}Ca -labelled incubation fluids containing dimethylsulphoxide (DMSO; final concentration = 4.2 $\mu\text{l ml}^{-1}$) without (○, ●) or with A23187 (□, ▲; final concentration = 10 μM), were added at zero time; the DMSO was used as a solvent for the ionophore stock solution. The protein concentration in the suspensions was 0.51 mg ml⁻¹. *b*, The ^{45}Ca -containing solutions were added at zero time. As indicated on the graph, DMSO (final concentration 2.5 $\mu\text{l ml}^{-1}$), without or with A23187 (final concentration = 10 μM), was added immediately after the 5 min samples were drawn. The protein concentration in the suspensions was 0.49 mg ml⁻¹.

experiments on rat liver and rat brain mitochondria. Figure 2 shows data from a representative experiment in which we directly compared Ca uptake by disrupted synaptosomes and by brain mitochondria (crude mitochondrial fraction "P₂" of Gray and Whittaker¹⁰). Note that, in the presence of the mitochondrial poisons, ATP significantly increased Ca uptake by the disrupted synaptosomes (Fig. 2d) but had no effect on Ca uptake by the P₂ fraction (Fig. 2c).

Several observations indicate that the ATP stimulates Ca uptake rather than superficial (surface) binding: (1) The extra ⁴⁵Ca on the filters is not displaced by the non-radioactive Ca (1.2 mM) in the wash solutions (all experiments). (2) There is no stimulation of Ca uptake by ATP, even after 7 min of incubation, if the incubation is carried out at 3 °C rather than at 30 °C (data not shown). (3) The divalent ion-specific ionophore, A23187, prevents the ATP stimulation of Ca uptake (Fig. 3a) and releases previously accumulated Ca (Fig. 3b). These data are consistent with the hypothesis that ATP induces "uphill" (that is, against an electrochemical gradient) transport of Ca into vesicular compartments¹¹. (4) The ATP-stimulated Ca uptake at long incubation times is enhanced when oxalate is present in the incubation medium (Fig. 4). If oxalate is passively distributed across the membranes of the Ca-accumulating particles, as appears to be the case in fragmented sarcoplasmic reticulum¹², the data in Fig. 4 imply that ATP promotes net transport of Ca into the vesicles so that

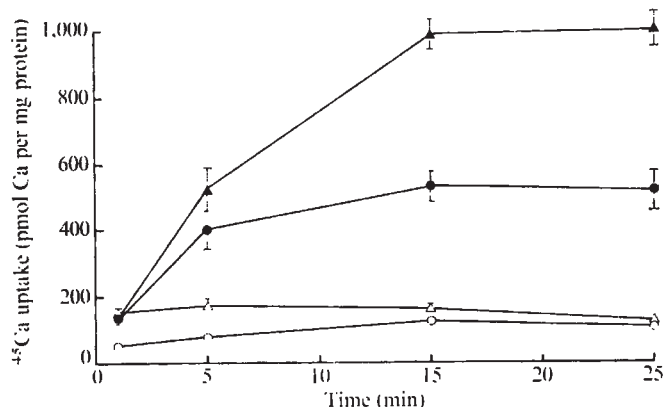


Fig. 4 Effect of oxalate on the time course of ⁴⁵Ca uptake by disrupted synaptosomes. Synaptosomes were suspended in 1.0 ml of lysis fluid (standard salt solution with NaCl omitted); after 1 min (time zero on the graph) ⁴⁵Ca-containing solutions (1.0 ml) were added to give a final Ca concentration of 5 μ M. The final composition of the incubation solutions was similar to the standard salt solution except that all of the NaCl was replaced either by 145 mM KCl (oxalate-free solutions; ○, ●) or by 5 mM K oxalate plus 137.5 mM KCl (△, ▲); all solutions contained 0.1 mM DNP, 0.1 mM Na azide and 0.7 μ g ml⁻¹ oligomycin. When 2 mM ATP was present (●, ▲), the MgCl₂ concentration was raised to 3 mM. Incubations were carried out at 30 °C; the protein concentration in the suspensions was 0.49 mg ml⁻¹. Each symbol is the mean of three determinations; error bars are shown where the standard errors range beyond the borders of the symbols.

the intravesicular Ca concentration rises and the calcium oxalate solubility product is exceeded. Atomic absorption measurements of total Ca (unpublished) indicate that ATP (in the presence of mitochondrial poisons) promotes net Ca accumulation by the particulate material in the disrupted synaptosome preparations; in the presence of ATP, oxalate increases the net uptake. (5) The effect of ATP on Ca uptake is greatly reduced in the absence of Mg (data not shown), perhaps indicating that a Mg-dependent ATPase may be involved in Ca sequestration¹³.

In summary, these experiments suggest that there are vesicular bodies, other than mitochondria, localised within presynaptic nerve terminals, which can accumulate Ca in the

presence of ATP and Mg. The properties of this transport system resemble those of fragmented sarcoplasmic reticulum¹¹⁻¹⁴. Similar non-mitochondrial ATP-dependent Ca sequestration has also been observed in whole brain microsomes^{15,16}; however this activity could not be assigned to the synaptic region because of the heterogeneity of the preparations.

Preliminary kinetic data indicate that the ATP-dependent Ca uptake by the disrupted synaptosome preparations has a half-saturation for Ca of less than 1 μ M; this sequestration mechanism may therefore operate at physiological intracellular Ca concentrations. By comparison, mitochondria apparently have a lower affinity for ionised Ca: in the presence of physiological K and Mg concentrations, half-saturation for Ca uptake by these organelles may be in excess of 10 to 20 μ M Ca (ref. 17). Thus, as an alternative to the hypothesis that mitochondria may be the main intraterminal Ca storage sites^{3,4}, our data are consistent with the idea that the mitochondria may help to buffer intraterminal Ca primarily by supplying ATP to the transport and storage system described above. This system may therefore play an important role in facilitation and post-tetanic potentiation because these phenomena seem to be the consequence of Ca retention by presynaptic nerve terminals^{18,19}.

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Chemical properties of human Ia antigens

THE human major histocompatibility complex encompasses at least four well-defined loci. Those called HLA-A, -B, and -C control the expression of the classical transplantation antigens (ref. 1). The fourth locus, HLA-D, controls determinants which regulate the ability to stimulate in mixed leukocyte cultures (MLR) and seems at least partly to correspond to the I-region of the murine H-2 complex. A great deal of work has been done on the immunogenetics of the HLA-D locus (reviewed in ref. 2) but despite the fact that alloantibodies against HLA-D determinants are available^{3,4} the molecular nature of the antigens is largely unknown. Such studies have been hampered by the fact that HLA-D determinants only

seem to be expressed on a small proportion of the peripheral blood lymphocytes (the B cells)⁵ but Fu *et al.*⁵ have reported data which suggest that B-cell-specific alloantigen is expressed on leukaemic cells. We have therefore isolated a cell membrane glycoprotein complex, controlled by the HLA-D locus, from leukaemic cells of a patient with chronic lymphatic leukaemia. The characteristics of the protein complex strongly suggest that it represents the human equivalent of the murine Ia antigens.

Patient P.S. suffering from chronic lymphatic leukaemia was investigated with respect to the HLA phenotype. Routine tissue typing with a panel of well-defined HLA alloantisera established the phenotype pattern to A1, A2, B8, Bw40, Cw3. The phenotypic pattern of the HLA-D locus was assessed with the MLR test with use of a panel of homozygous typing cells⁶. A clearly positive response for Dw3 was obtained but no other HLA-D specificity could unambiguously be assigned to the patient. Additional testing with HLA-D alloantiserum-induced cytotoxicity⁷ on the leukaemic cells strongly suggested that the HLA-D phenotype was Dw3 and Dw4.

An alloantiserum obtained from a multiparous woman strongly inhibited the MLR response when the stimulating cells were of the Dw3 phenotype. No effect of the antiserum was noted when lymphocytes of a Dw3 homozygous donor were used as the responder cells and the stimulator cells were of another HLA-D type. The antiserum was as effective in impeding the MLR response even after absorption on platelets of the relevant HLA type, but after absorption, the antiserum was only cytotoxic for B lymphocytes of the Dw3 donor origin. Another alloantiserum which blocked the MLR response stimulated by cells expressing Dw4, was rendered specific for the HLA-D locus determinants by the same procedure.

Leukocytes of patient P.S. were obtained by leukocytapheresis and the lymphocytes were isolated by Ficoll-Isopaque gradient centrifugation⁸. Cell-surface proteins of the isolated cells were labelled by lactoperoxidase-catalysed iodination and Triton X-100-solubilised molecules reactive with the alloantisera directed against Dw3 and Dw4 determinants were precipitated and analysed by SDS-polyacrylamide gel electrophoresis according to a procedure detailed elsewhere⁹ (Fig. 1). It is evident that both antisera precipitated two types of polypeptide chains with apparent molecular weights 28,000 and 34,000. No molecules of the HLA-type, that is with apparent molecular weights 12,000 and 45,000, were observed suggesting that the absorption of the antisera had been successful. The specificity of the antisera was also ascertained by the fact that normal human serum did not react with either of the two types of polypeptide chains (Fig. 1c). Moreover, absorption of the alloantiserum against Dw3 determinants on Dw3-expressing homozygous cells completely abolished its reactivity with the two polypeptide chains. On the other hand, absorption of this alloantiserum on Dw4-expressing homozygous B lymphocytes did not diminish its reactivity with the two polypeptide chains from Dw3-expressing cells.

In other experiments leukaemic cells of patient P.S. were cultured in the presence of ³H-leucine and H-fucose, respectively⁹. Radiolabelled molecules reactive with alloantisera against Dw3 and Dw4 determinants were isolated and analysed by SDS-polyacrylamide gel electrophoresis. With either of the radioactive labels the two polypeptide chain pattern was evident. Consequently, both polypeptide chains contain carbohydrate moieties.

Since the SDS-polyacrylamide gel electrophoretic analyses were performed in reducing conditions, we looked to see whether the two types of polypeptide chains might be associated by disulphide bonds. In the absence of reduction the two polypeptide chains were separated and, thus, no covalent interaction was apparent (Fig. 1f). The

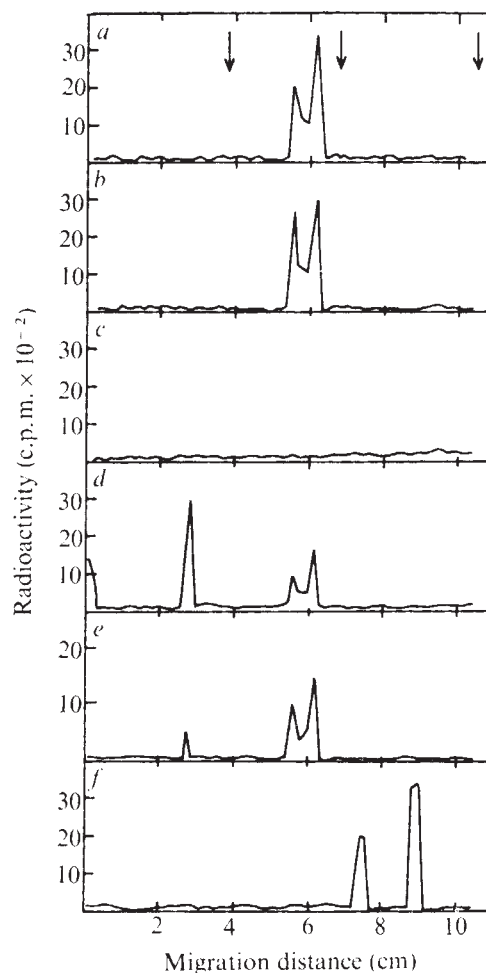


Fig. 1 SDS-polyacrylamide gel electrophoresis under reducing conditions of Triton X-100-solubilised, ¹²⁵I-labelled membrane antigens reactive with alloantisera directed against HLA-Dw3 (a) and HLA-Dw4 (b) determinants. No molecules reacted with a normal human serum (c). ¹²⁵I-labelled membrane molecules, crosslinked with TDH, were precipitated by a sandwich procedure using an alloantiserum directed against Dw3 determinants and analysed by SDS-polyacrylamide gel electrophoresis (d). The component with the apparent molecular weight 60,000 was extracted from the gel, treated with 15 mM NaIO₄ to cleave the crosslinker, and resubjected to SDS-polyacrylamide gel electrophoresis (e). The same material as in a was analysed by SDS polyacrylamide gel electrophoresis in the absence of reducing agents (f). The arrows denote the migration positions (from left to right) of marker heavy and light chains of IgG and the marker dye bromophenol blue. (a-e). The markers in (f) were immunoglobulin light chains (molecular weight 23,000, migration distance 10.2 cm), ovalbumin (molecular weight 43,000, migration distance 6.0 cm) and bovine serum albumin (molecular weight 68,000, migration distance 3.0 cm).

two polypeptide chain pattern may result, however, from the existence of non-covalent interactions between the chains in physiological conditions. Alternatively, the alloantisera may react with two independent molecules. To resolve this question Triton X-100-solubilised, ¹²⁵I-labelled membrane proteins were crosslinked with the cleavable crosslinker tartryl-di(glycylhydrazine) (TDH)¹⁰ and reacted with the alloantiserum against Dw3. Three types of molecules were observed after SDS-polyacrylamide gel electrophoresis (Fig. 1d). In addition to the two usually encountered polypeptide chains a new component with the apparent molecular weight 60,000 was evident. The latter was extracted from the gel, the crosslinker cleaved by treatment with NaIO₄ (ref. 10), and the material resubjected to SDS-polyacrylamide gel electrophoresis. The result (Fig. 1e) demonstrates that the 60,000-dalton component most probably comprised one polypeptide chain of

each type, held together in the denaturing conditions by the crosslinker.

An intriguing observation is that the smaller of the two HLA-D chains always contains more radioactivity than the larger chain regardless of whether external or internal labelling has been used. This result may suggest that the small chain contains more of the labelled residues (leucine, tyrosine, and fucose) than the large chain. Alternatively, the small chain may have a greater turnover than the large chain and consequently may incorporate more of the radioactive precursors. In addition, the small chain could conceivably be more exposed on the membrane surface than the large chain which would account for the results obtained with lactoperoxidase-catalysed iodination (compare ref. 13). Further studies are needed to resolve these possibilities.

Further evidence for a firm non-covalent interaction between the two types of polypeptide chain was obtained from molecular weight determinations in non-denaturing conditions. A large scale isolation of material reactive with the anti-Dw3 alloantiserum was performed. From crude cell membranes of about 200 g of leukaemic cells (expressing the HLA-D specificities Dw3 and Dw4) sodium deoxycholate-solubilised molecules were purified by successive fractionation steps involving affinity chromatography on lectin columns and gel chromatography¹¹. Of the material in the most highly purified fraction about three-quarters reacted with the HLA-D alloantisera whereas the remainder of the material comprised unrelated molecules. Part of the HLA-D reactive material in this fraction was labelled with ¹²⁵I and subjected to analytical gel chromatography and sucrose density gradient centrifugation in deoxycholate. The distribution of molecules expressing the allotypic determinants was assessed by indirect immunoprecipitation. The molecules reactive with the alloantisera were eluted earlier than marker HLA molecules and they also sedimented faster than the HLA antigens. The Dw3-expressing molecules were analysed by SDS polyacrylamide gel electrophoresis and the peak fractions always displayed patterns similar to those shown in Fig. 1a and b. From the values of the Stokes' radius (44 Å) and the sedimentation constant (4.4S) a molecular weight for Dw3 and Dw4-expressing molecules of about 83,000 could be calculated¹². This molecular weight represents the sum of the weights of the individual polypeptide chains and the bound detergent. Since each one of the two types of polypeptide chains seems to bind an appreciable amount of detergent, but displays much lower values on sedimentation velocity analyses than the intact component (L.S.-T. unpublished observations) it seems likely that the 83,000 dalton complex comprises the two polypeptide chains in a 1:1 ratio and about 20,000 daltons of bound detergent.

The present data do not formally exclude the possibility that the glycoprotein complex is composed of two identical polypeptide chains which are very susceptible to proteolysis. No molecules comprising chains of a single type have ever been observed however, and in ³H-leucine labelling experiments the label occurs simultaneously in the two chains. This strongly suggests that the two types of chain are not involved in a precursor-product relationship.

The data reported above suggest that the isolated components may be the human equivalents of the murine Ia antigens (see ref. 13). To investigate this problem in some detail we have raised a rabbit antiserum against the protein complex. Fab fragments of these antisera abolished the lymphocytotoxic action of all HLA-D alloantisera tested and blocked the MLR response when bound to stimulator but not to responder cells in the MLR (L.K., unpublished observations and Ken Welsh, personal communication). These antisera were specific for the HLA-D determinants of normal peripheral blood lympho-

cytes as revealed by indirect immunoprecipitations and SDS-polyacrylamide gel electrophoresis (Fig. 2), and after solubilised membrane molecules had been reacted with the heteroantisera no molecules remained in the supernatant which bound to the relevant HLA-D alloantisera. One of the antisera (from Ken Welsh) reacted weakly but significantly with ³H-leucine-labelled B10.S splenocyte membrane molecules and, as can be seen in Fig. 2, the polypeptide chain pattern on SDS-polyacrylamide gel electrophoresis is that of typical murine Ia antigens. The heteroantisera did not cross react with HLA antigens derived from the A, B and C loci. Neither did heteroantisera directed against the A, B and C locus gene products¹ react with the glycoprotein complex. This suggests that there is no apparent immunological similarity between the molecules coded for by the D locus and those coded for by the A, B and C loci.

The present data in conjunction with previous reports¹⁵⁻¹⁷ strongly suggest that the HLA-D locus controls the expression of polypeptide chains which are the equivalents of the murine Ia antigens. Like the latter molecules the HLA-D-derived antigens are composed of two types of glycoprotein chains exhibiting immunological cross reactivity with murine Ia antigens which in non-denaturing

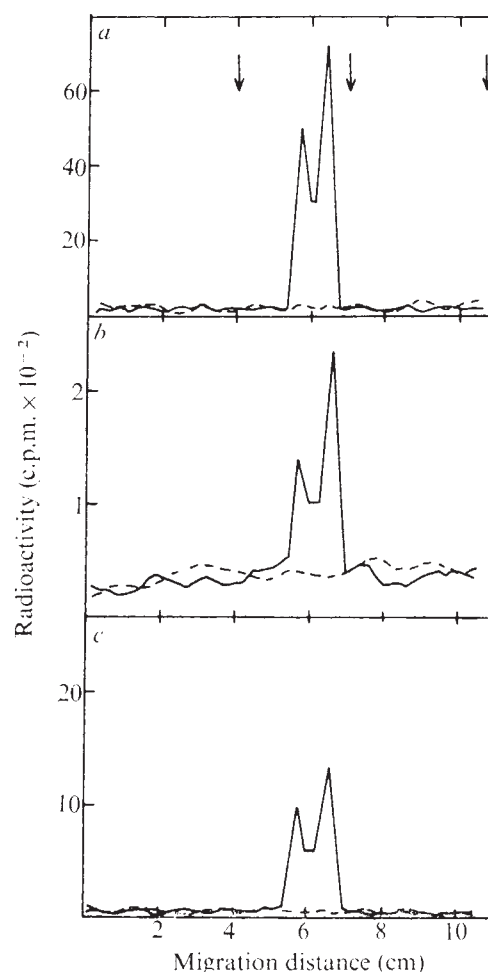


Fig. 2 SDS-polyacrylamide gel electrophoresis of Triton X-100 solubilised, ¹²⁵I-labelled membrane antigens of lymphocytes from normal peripheral blood reactive with a rabbit antiserum directed against the 83,000-dalton glycoprotein complex (a). In (b) the same antiserum was reacted with ³H-leucine-labelled B10.S splenocyte macromolecules, and in (c) the rabbit antiserum was substituted for an A.TL anti-A.TH serum (anti-Ia^b). In a, b, and c the broken curves represent molecules reactive with normal rabbit serum (a and b) and normal mouse serum (c). The arrows denote the migration positions (from left to right) of marker heavy and light chains of IgG and the marker dye bromphenol blue.

conditions form a stable complex comprising one chain of each type. The protein protein association seems to involve non-covalent interactions only. Available information does not demonstrate if both polypeptide chains express allo-antigenic determinants. It is therefore possible that only one of the chains, as for the HLA antigens¹⁴, is controlled by the HLA region. Studies in progress are aimed at elucidating this question.

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Recognition of a naturally occurring idotype by autologous T cells

BONE-MARROW-derived (B) lymphocytes belonging to the same clone bear antigen receptors which share a specific marker (idiotype) with antibodies secreted by their progeny¹. According to the network theory of immunity², immuno-competent B cells recognise each other through a network of idiotypic anti-idiotypic interactions. This theory implies the existence of B and T cells specific for autologous idiotypes. To facilitate detection of such cells it is advantageous to study an immune response of restricted heterogeneity. The immune response of BALB/c mice to phosphorylcholine (PC) is idiotypically homogeneous: the anti-PC antibodies produced bear almost exclusively the idiotypic characteristic of the BALB/c PC-binding myeloma protein TEPC-15 (T15) (ref. 3). T15 is a member of a family of PC-binding myeloma proteins of BALB/c origin, which bear distinct idiotypes⁴. Idiotypes representative of these other proteins are not, however, expressed by BALB/c on immunisation with PC. It has recently been reported that helper T cells from BALB/c mice can discriminate among idiotypes expressed on several of these anti-PC myeloma proteins. These authors did not, however, demonstrate

helper T cells specific for the T15 idiotypic⁵. This failure was attributed to a state of tolerance induced and maintained by the high levels of immunoglobulin bearing the T15 idiotypic in conventional BALB/c mice. In this report we demonstrate the existence of T cells reactive to an autologous idiotypic and we discuss the possible regulatory role of these T cells in the immune response to PC.

To generate idiotypic-specific T cells, conventional and neonatally suppressed BALB/c mice were immunised with T15. Neonatal suppression was accomplished by injecting 0.1 ml of A/J anti-T15 serum at birth as previously described⁶. Mice received a total of 400 µg of protein in a series of four injections over a period of 2 weeks. The first injection was given in complete adjuvant in the foot pads, the second was given in incomplete adjuvant, intraperitoneally (i.p.). The final two injections were given in saline i.p. At least four weeks after the last injection mice were sacrificed and T cells were purified from both spleen and pooled lymph nodes using the method of nylon wool filtration⁷. Dinitrophenyl (DNP)-specific B cells were obtained from BALB/c mice immunised with 100 µg of alum-precipitated DNP conjugated keyhole limpet haemocyanin (DNP₉-KLH) and 10⁶ *Bordetella pertussis* organisms. These mice were rested for at least 6 weeks before sacrifice, when their spleen cells were treated with anti-Thy 1.2 serum and complement.

The T15 primed T cell populations were assayed for helper cell activity in a hapten-carrier adoptive transfer system with DNP-primed B cells and DNP-conjugated T15 (DNP₉-T15) as antigen. To demonstrate the specificity of the T15 primed T cells, helper activity was assessed in the same transfer system with DNP-conjugated McPC603 (DNP₉-M603) as antigen. M603 is another myeloma protein of BALB/c origin with binding activity for phosphorylcholine and differs from T15 only in its idiotypic specificity^{8,9}. Conjugation of either T15 or M603 with DNP did not alter the idiotypic determinants which characterise these two myelomas (results not shown). Therefore, these idiotypes should be available as carrier-specific determinants.

DNP-primed B cells were transferred with T15-primed T cells into conventional recipients irradiated with 600 r. Each recipient received 50 µg of aqueous DNP₉-T15 along with the cells. The animals were sacrificed 8 d later and their spleens assayed for indirect anti-DNP plaque-forming cells (PFC) using the Cunningham and Szentgyörgyi¹⁰ modification of the haemolytic plaque technique¹¹. DNP-conjugated sheep erythrocytes prepared by the method of Inman *et al.*¹² were used as indicator cells.

Table 1 shows that when 3×10^6 DNP-primed B cells were transferred with 10×10^6 T15-primed splenic T cells obtained from suppressed animals, the resulting response was 2,340 indirect anti-DNP PFC per spleen. This response is 15-fold higher than the background response of 142 anti-DNP PFC calculated by summing the responses obtained on transfer of the same cell populations separately. Lymph node T cells from the same T15-primed suppressed animals did not provide any detectable helper activity above background. In addition, neither splenic nor lymph node T cells obtained from T15-primed conventional donors provided as efficient helper activity as splenic T cells obtained from T15-primed suppressed donors; although transfer of either of the non-suppressed T cell populations with DNP-primed B cells did result in an anti-DNP PFC response 6-fold higher than background (Table 1).

Since conventional BALB/c mice have high levels of circulating immunoglobulin (Ig) bearing the T15 idiotypic⁸, these molecules might compete with DNP₉-T15 and inhibit the helper activity of T15 specific T cells. To optimise the expression of the T15 specific helper cells we used irradiated (600 r) neonatally suppressed⁶ BALB/c mice as recipients. These animals contained less than 0.68 µg ml⁻¹ of circulating Ig bearing T15 idiotypic compared with conventional BALB/c mice which contain between 40–80 µg ml⁻¹.

Table 1 Demonstration of idiotype specific helper T cells

DNP-KLH [†] primed B	Number of cells transferred ($\times 10^6$) [*]				Indirect anti-DNP PFC/spleen ($\times 10^3$) [¶]
	Suppressed T15 primed [‡] spleen T	lymph node T	Conventional T15 primed [§] spleen T	lymph node T	
3	—	—	—	—	0.49 (0.30–0.78)
—	10	—	—	—	0.93 (0.66–1.32)
—	—	10	—	—	0.68 (0.30–1.51)
—	—	—	10	—	< 0.4
—	—	—	—	10	< 0.2
3	10	—	—	—	23.4 (13.8–39.8)
3	—	10	—	—	2.9 (2.0–4.3)
3	—	—	10	—	5.4 (3.4–8.6)
3	—	—	—	10	5.0 (2.9–8.9)

*Mixtures of cells were transferred into 600 r irradiated conventional BALB/c mice intravenously. Animals received 50 μ g of aqueous DNP₆-T15 simultaneously with transferred cells. Animals were sacrificed 8 d after cell transfer.

[†]DNP-KLH primed spleen was treated with anti-Thy 1.2 serum and complement resulting in a population containing 94% immunoglobulin bearing cells and 2% T cells as assessed by immunofluorescence.

[‡]Suppressed BALB/c donors were given 0.1 ml of A/J anti-T15 serum intraperitoneally at birth. At 2 months of age suppressed animals were immunised with two injections of 100 μ g of TEPC15 per week for 2 weeks. T cells were isolated from spleen and pooled lymph nodes using nylon wool fractionation. The purified splenic T population contained 93% T cells and 1.8% immunoglobulin-bearing cells and the lymph node T population contained 97% T cells and 1.4% immunoglobulin-bearing cells (as assessed by immunofluorescence).

[§]Conventional BALB/c mice were immunised with TEPC15 as the suppressed animals. Splenic T cells and pooled lymph node T cells were purified using nylon wool. The resulting spleen T-cell population contained 90% T cells and 1% immunoglobulin-bearing cells and the lymph node T-cell population contained 95% T cells and 0.6% immunoglobulin-bearing cells (as assessed by immunofluorescence).

[¶]Geometric mean of the responses of 4 or 5 animals per group. Numbers in parentheses represent one s.e. about the mean. The direct PFC response in all groups was less than 5% of the total response. Indirect PFC were developed using a polyvalent rabbit anti-mouse immunoglobulin serum.

When 2×10^6 or 10×10^6 splenic T cells obtained from T15 primed suppressed mice were transferred along with 3×10^6 DNP primed splenic B cells into suppressed recipients, the resulting anti-DNP PFC responses were 50-fold and 20-fold higher than background, respectively (Table 2). These T cells are specific for the T15 idiotype since transfer of the same cell population with DNP₆-M603 as antigen did not result in an anti-DNP response above background (Table 2).

T cells can be induced in conventional BALB/c but not as readily as in suppressed mice.

Splenic T cells isolated from suppressed unprimed BALB/c and transferred along with DNP-primed B cells into suppressed irradiated recipients, failed to give an anti-DNP response significantly above background (data not shown). Thus, if mature T15-specific helper T cells exist in unprimed suppressed BALB/c, there are too few to be detected and/or antigen (T15)-dependent proliferation and maturation of

Table 2 Enhanced expression of idiotype specific helper activity in idiotype suppressed recipients*

DNP-KLH [†] primed B	Number of cells transferred [‡] (× 10 ⁶) Suppressed T15 Primed [§] Spleen T	Antigen	Indirect anti-DNP PFC per spleen (× 10 ³) [¶]
3	—	DNP ₆ -T15	3.1 (2.4-4.1)
—	20	DNP ₆ -T15	4.3 (2.6-6.9)
3	20	DNP ₆ -T15	376.0 (301-469)
3	10	DNP ₆ -T15	151.0 (118-193)
3	10	DNP ₆ -M603	8.5 (5.0-14.5)

*All recipients were injected with 0.1 ml of A/J anti-T15 serum intraperitoneally at birth. Recipients were 3 months of age at time of transfer and contained $< 0.68 \mu$ g ml⁻¹ of Ig sharing idiotypic determinants with T15.

[†]Mixtures of cells were transferred into 600 r irradiated idiotype-suppressed BALB/c mice with 50 μ g of aqueous antigen. Animals were sacrificed 8 d after transfer.

[‡]DNP-KLH primed spleen cells were treated with anti-Thy 1.2 serum and complement resulting in a population containing 93% immunoglobulin bearing cells and 0.7% T cells as assessed by immunofluorescence.

[§]Spleen T cells were isolated from idiotype-suppressed TEPC15-primed BALB/c mice; see legend to Table 1. The resulting population contained 93% T cells and 1.2% immunoglobulin-bearing cells as assessed by immunofluorescence.

[¶]Geometric mean of the responses of 3 or 4 animals per group. Numbers in parentheses represent 1 s.e. about the mean. The direct anti-DNP PFC response in all groups was less than 5% of the total response. Indirect PFC were developed using a polyvalent rabbit anti-mouse immunoglobulin serum.

The greater efficiency with which T15-specific helper activity is generated in neonatally suppressed compared with conventional BALB/c mice is perhaps related to the fact that suppressed mice have 100-fold lower concentrations of circulating Ig bearing the T15 idiotype⁶. The high levels of T15 idiotype in conventional BALB/c might partially mask the T15-specific T-cell clones. Consistent with this hypothesis is the finding that T15-specific B cells are also more readily induced in suppressed than conventional BALB/c mice on immunisation with T15 (H.C., A.A. and M.H.J., in preparation). The ability of conventional mice to produce anti-T15 antibodies demonstrates that the T15-specific precursor cells are present but simply not expressed to the same extent as in suppressed mice. Similarly, T15-specific

T15-specific precursor T cells might be required for generation of helper activity.

Idiotype-specific T and B cells might play a part in regulation of the immune response to PC. We have recently demonstrated that BALB/c mice can produce antibodies specific for the T15 idiotype. These autologous anti-idiotypic antibodies modulate the idiotypic pattern of the immune response to PC *in situ*, that is, the proportion of antibodies produced which bear the T15 idiotype is reduced compared with normal controls (H.C., A.A. and M.H.J., in preparation). The production of these antibodies is T dependent since 'B mice' (adult thymectomised, lethally irradiated and foetal liver reconstituted) fail to produce anti-T15 antibodies on immunisation with T15 (M.H.J., unpublished).

Although we have shown that idiotype-specific T cells can function as helpers in the production of anti-DNP antibodies, these T cells might also collaborate with B cells of the same specificity in the production of autologous anti-T15 antibodies. Alternatively, analogous to the studies of Eichmann¹², T15-specific T cells could function as suppressor cells and directly regulate the expression of PC-specific B cells expressing receptors bearing the T15 idiotype. We are currently investigating the modes of action of T15-specific T cells in an attempt to define their possible role in regulation of the anti-PC response.

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Altered expression of human monocyte Fc receptors in malignant disease

SEVERAL kinds of cells involved in immunity can bind antibody or immune complexes through surface receptors which interact with the Fc portion of IgG (Fc receptors). The expression of Fc receptors on mononuclear phagocytes (monocytes/macrophages) can undergo changes in response to various stimuli both *in vitro*¹ and *in vivo*²⁻⁴ and such changes may play a regulatory role in the induction of an immune response and in immunological effector mechanisms. Here I show that the expression of Fc receptors on circulating human monocytes is substantially increased in individuals with solid malignant tumours. This change seems not to be a normal characteristic of infectious or inflammatory diseases of a non-malignant nature.

The experimental group consisted largely of individuals with various bronchogenic carcinomas (squamous cell, oat cell, adenocarcinoma) and included examples of carcinoma of the rectum, pharynx, oesophagus and breast. All were untreated and the majority were without signs of metastatic disease. Control subjects were healthy laboratory workers and were initially age and sex matched with patients until it became evident that significant age and sex differences were not present amongst normal donors. In the study of non-malignant diseases individuals with the most active disease were selected where possible, thus for example, all pulmonary tuberculosis patients had live bacilli in their sputum.

Lymphocytes and monocytes were isolated from 10 ml of heparinised peripheral blood by centrifugation over a Ficoll/trisil gradient. The cells were washed twice, adjusted to approximately 10^6 ml⁻¹ in Hank's balanced salt solution (BSS) and 1-ml aliquots of this were placed in each chamber of dual tissue culture slides (Lab-tek products). After incubation at 37 °C for 90 min the glass adherent cells were washed three times with BSS. This

procedure resulted in a monolayer consisting of monocytes and a proportion of quite firmly adherent lymphocytes. The former were easily distinguishable from lymphocytes because of the spreading which occurred following monocyte adherence. However, this morphological identification was confirmed by latex particle ingestion⁵.

Fc receptor expression was characterised using an assay similar to that described for guinea-pig macrophages⁶. A panel of erythrocytes, bearing increasing amounts of specific antibody, was prepared by incubating a 1% suspension of ox erythrocytes with heat-inactivated rabbit anti-ox antiserum at increasing concentrations in phosphate-buffered saline. After 30 min at 20 °C the cells were washed once and resuspended in BSS at a concentration of 1%. One-millilitre aliquots of sensitised erythrocytes were added to each monolayer and allowed to settle for 1 h at 20 °C. The monolayer were then washed five times with BSS, fixed with 1% glutaraldehyde and stained with buffered Giemsa. Mounted preparations were examined and cells with five or more attached erythrocytes were scored as rosettes. The percentage of rosette-forming monocytes occurring at each increment of erythrocyte sensitisation in a monolayer from a normal donor was compared with that occurring simultaneously in a monolayer from a donor with malignant disease. In the study of non-malignant diseases the comparison between monocytes from

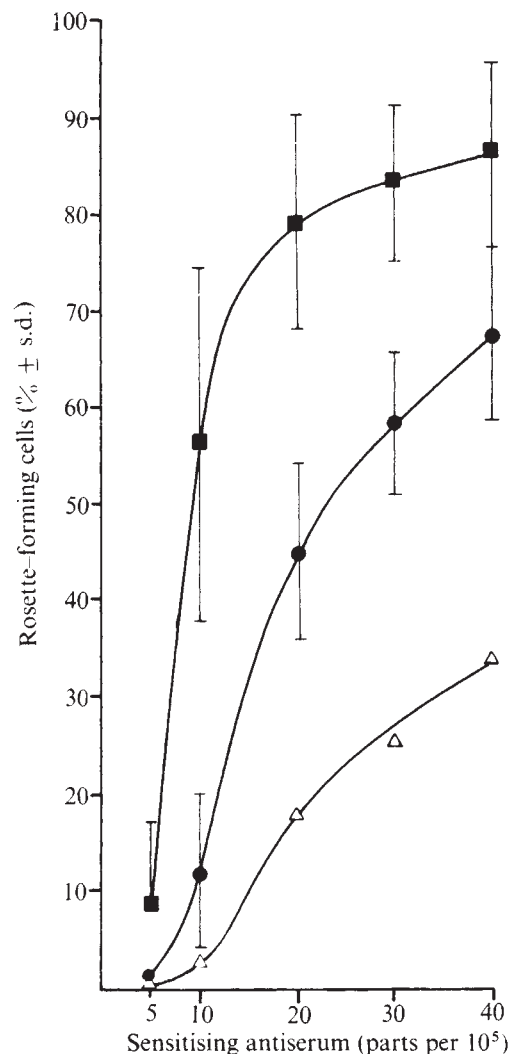


Fig. 1 The percentage of rosette forming monocytes as a function of the degree of erythrocyte sensitisation in fourteen individuals with malignancies (■), in seventeen normal individuals (●), and in one individual with malignant disease (lung cancer) and maturity-onset diabetes (△). Values are expressed as means $\pm$ one standard deviation.

Table 1 Occurrence of B lymphocytes, T lymphocytes and T lymphocytes expressing Fc receptors (FcR), as judged by rosette formation, in peripheral blood of individuals with malignant tumours

	Non-T lymphocytes expressing FcR (B lymphocytes and others) % of total	T lymphocytes % of total	T lymphocytes expressing FcR % of total
JC	36.0	64.0	2.5
TJ	35.0	65.0	5.0
MC	35.5	64.5	7.0
RH	39.0	61.0	6.0
SF	35.0	65.0	3.0

Lymphocytes isolated from 10 ml of heparinised peripheral blood on Ficoll/trisil gradients were assayed by the method of Haegert *et al.*⁵. Cells expressing FcR bind ox erythrocytes sensitised at an optimal concentration of antiserum (60 parts per 10³) while T lymphocytes bind fluorescein-labelled sheep erythrocytes. Any T lymphocytes expressing FcR bind both indicator cells.

patients and from healthy donors was made using those two increments of sensitisation which had been established as optimal for detecting differences in receptor expression. Fc receptor expression by lymphocytes from a group of patients with untreated bronchogenic carcinoma was also characterised by a mixed rosette suspension assay in which T lymphocytes bound fluorescein-labelled sheep erythrocytes and B lymphocytes, and any other cells expressing Fc receptors, bound antibody-sensitised ox erythrocytes⁵.

The dose-response curves in Fig. 1 show monocyte rosette formation as a function of the degree of erythrocyte sensitisation. At the second increment the proportion of monocytes binding immune complexes in patients with malignant disease is five times greater than normal. Fourteen of the fifteen patients tested showed a substantial increase in binding at all increments of sensitisation indicating a uniform increase in Fc receptor expression rather than a change in the proportion of high avidity cells¹. The one patient who did not exhibit elevated receptor expression showed instead a marked depression in comparison with normal (Fig. 1). This bronchogenic carcinoma patient also had maturity-onset diabetes mellitus and was receiving chlorpropamide at the time of sampling.

Insulin inhibits the differentiation of macrophage Fc receptors *in vitro*⁶ and a similar effect might explain this *in vivo* observation since higher than normal levels of insulin occur in maturity-onset diabetes⁷. It was not possible to demonstrate any increase in the expression of Fc receptors by T lymphocytes in patients with bronchogenic carcinoma. Very few T cells expressing Fc receptors were observed using the mixed rosette suspension assay of Haegert and associates⁵ (Table 1).

The expression of Fc receptors by monocytes from individuals with non-malignant diseases is compared with that of normal donors in Table 2. Each datum is the largest difference observed in percentage rosette formation between a monolayer from the patient and a monolayer from a normal donor assayed simultaneously. Significant differences (greater than 20%) are boxed in for clarity and examples of malignant disease are included for comparison. Significant differences from normal do not seem to occur in the non-malignant diseases tested with the interesting exception of a proportion of patients with active pulmonary tuberculosis (three out of seven). Also, one patient with a surgical wound infection exhibits a significant difference while one patient with a breast lump later found to be malignant does not show significantly altered receptor expression.

Mononuclear phagocytes, in a variety of experimental systems, have been shown to exert a regulatory effect on the function of lymphocytes (reviewed by Nelson⁸). Macrophages seem to release both stimulatory and inhibitory factors which act on lymphocytes⁹ while the macrophage surface may adsorb both cooperative¹⁰ and suppressive¹¹ factors released by lymphocytes. In studies of the apparently complex relationship between macrophage and lymphocyte function one of the more consistent observations has been the suppression of T lymphocyte responses by activated macrophages⁸. In a study particularly pertinent to the present report Berlinger and associates showed that peripheral blood monocytes of patients with malignant tumours exert an inhibitory effect on the blastogenic response of the patients' lymphocytes in mixed

Table 2 Monocyte Fc receptor expression in non-malignant diseases

	No. of cases							
Pregnancy (week 32)	4	+12	+18	+7	+14			
Benign breast tumours	3	+3	+7	+12				
Rheumatoid arthritis	5	-7	+6	+14	+16	-17		
Gonorrhoea	6	-10	-3	-2	+8	-17	+3	
Pulmonary tuberculosis	7	-16	-11	-48*	+4	-53*	+37*	5
Chronic bronchitis	5	-4	-14	+4	1	-4		
Acute bronchitis	1	-1						
Bronchiectosis	1	-5						
Asthma (extrinsic)	2	-8	-17					
Viral meningitis	2	-11	-1					
Surgical wound infections	6	-11	+14	+42*	+1	+7	+10	
Dermatitis	4	-12	+6	-10	+13			
Squamous cell carcinoma of bronchus	(e.g.)	+66*						
Adenocarcinoma of bronchus	(e.g.)	+39*						
Carcinoma of rectum	(e.g.)	+63*						
Carcinoma of breast stage II	1	+35*						
Carcinoma of breast stage I	1	+9						

Monolayers were assayed using indicator cells sensitised with rabbit anti-ox antiserum at a concentration of 10 and 20 parts per 10³. Each datum represents the largest difference in percentage rosette formation observed between a monolayer from a patient and from a normal donor assayed simultaneously. Significant differences are marked with an asterisk and examples of malignant disease are included for comparison.

leukocyte cultures¹². The present study shows that altered expression of monocyte Fc receptors occurs in malignant disease but does not seem to occur readily in a variety of non-malignant diseases. This change in monocyte membrane characteristics may be an appropriate host response, facilitating macrophage-mediated cytotoxicity and the clearance of immune complexes, and tumour progression may occur because of lesions elsewhere in the host's defence system¹³. An alternative possibility is that the change in monocyte surface characteristics, or associated changes, occurring in malignant disease exert a net inhibitory effect on anti-tumour immunity. This might occur locally rather than systemically during the early growth of a tumour. The same macrophage-mediated inhibition of an immune response might also occur in chronic infections such as tuberculosis.

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Serological evidence for H-Y antigen in *Sxr*, XX sex-reversed phenotypic males

It is generally considered that the Y chromosome is necessary for the development of the mammalian testis and the consequent male phenotype. Exceptions occasionally occur in man and other animals, where a male phenotype is associated with an apparently normal female karyotype. In some instances it has been possible to show that such "sex reversal" has an autosomal genetic basis. Although this might imply that the Y chromosome is not a prerequisite of maleness, it has not been excluded that a small male-determining region of the Y may have been translocated to an autosome without producing a recognisable karyotypic change¹ (reviewed in ref. 2). The best studied case of male development with an apparently female karyotype is provided by the *Sxr* mutant in the mouse. This autosomal dominant condition causes both XX and XO mice to develop as males, although germ cells

are absent in *Sxr*,XX animals and spermatogenesis is impaired in *Sxr*,XO mice. *Sxr*,XY males have a slightly reduced testis size but are usually fertile. Cytological scrutiny of mitotic and meiotic chromosomes (including chromosome banding) has revealed no evidence for a Y-autosome translocation, although a small translocation undetectable by the methods used cannot be ruled out (reviewed in ref. 2). This situation can now be investigated using serological methods for detecting H-Y (histocompatibility-Y) antigen on the surface of mouse sperm³ and male epidermal cells⁴. These techniques have provided further evidence that expression of this antigen is governed by a gene (or genes) on the Y chromosome even in the absence of the normal male phenotype⁵. Thus, serological demonstration of H-Y antigen offers a non-karyological criterion for the presence of a translocated portion of the Y in *Sxr*,XX males, because their cells should express H-Y if *Sxr* is a translocation involving the H-Y genetic determinant. We report here that *Sxr*,XX males express H-Y to a degree that may be the same or slightly lower than that of normal males, and that their *Sxr*,XY siblings seem to have near-normal levels of H-Y. The simplest explanation is that male-determining and H-Y-determining portions of the Y chromosome are present in the animals carrying *Sxr*.

The *Sxr* stocks were obtained from Harwell where they are maintained on a mixed C3H/HeH-101/H genetic background with tabby (*Ta*) being used as an X-chromosome

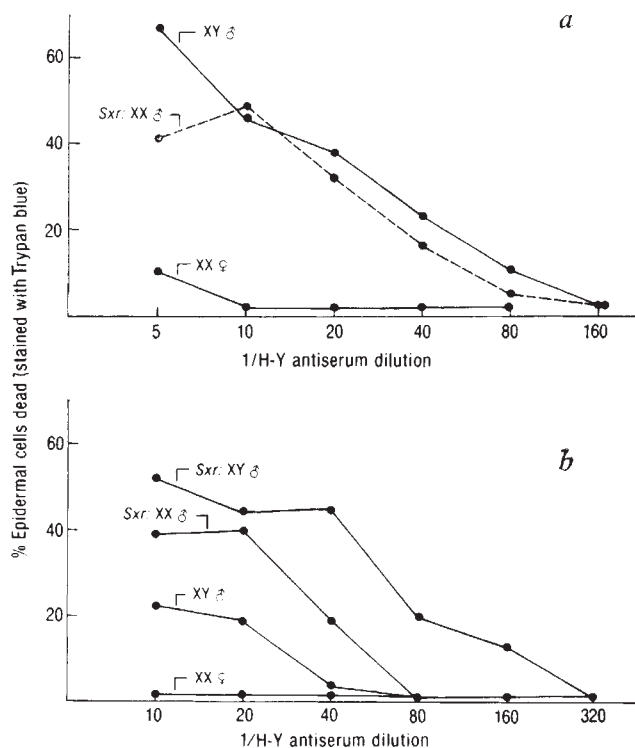


Fig. 1 Demonstration of H-Y antigen on dissociated epidermal cells of *Sxr*,XX and *Sxr*,XY mice by the complement-dependent cytotoxicity assay with anti-H-Y serum. *a*, The data in this figure are from a representative test with two animals in each group. The values shown are mean readings for the pairs of (C3H × 101)F₁ outcross stock controls and for the *Sxr*,XX individuals. Antiserum and complement were the same as indicated in Fig. 1 of ref. 3. Percentage of epidermal cells dead (ordinate) was calculated as indicated in the legend to that figure. *b*, The data in this figure are from one of two sets where *Sxr*,XY males were compared with their sibling XY (non-carrier) males. The two classes of XY male siblings were distinguished on the basis of testis weight. Although this criterion does not provide absolute assurance of genotype, we identified putative *Sxr*,XY animals by testis weight in the extreme low range (0.069 g and 0.078 g) compared with XY males with testis weight in the extreme high range (0.102 g and 0.127 g). The siblings were selected from single litters and were therefore the same age at the time of the test.

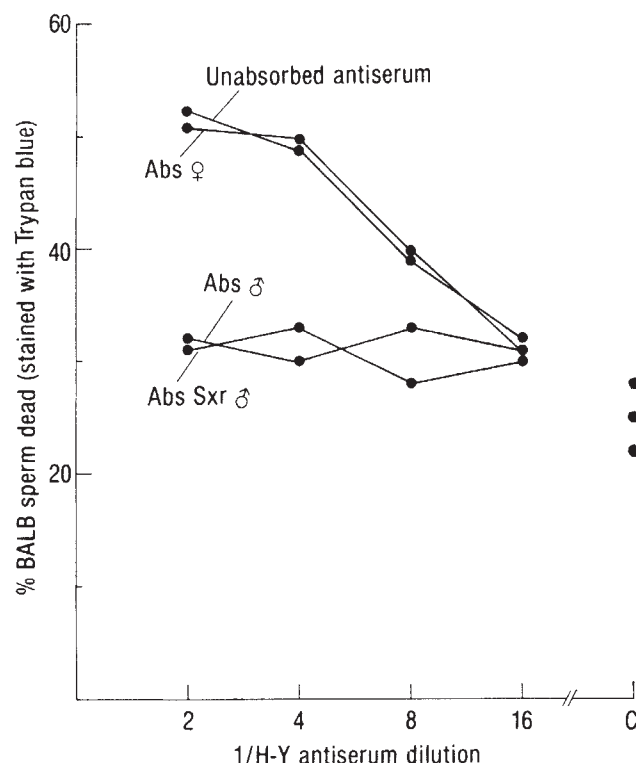


Fig. 2 Summary of cytotoxicity tests on mouse sperm with mouse H-Y antiserum after absorption with liver cells from normal females (B6), normal males (B6) and *Sxr,XX* males (see text). Abs, absorption with cells from the indicated sex. Each point represents an average value from three separate tests. C, control (complement included, antiserum omitted). Sperm suspensions were scored as coded samples. Tests were performed with BALB epididymal sperm according to Goldberg *et al.*³, using serum raised in C57BL/6 (B6) female mice against B6 male spleen cells. Equal volumes (0.05 ml) of H-Y antiserum, sperm (5×10^6 per ml), and diluted rabbit serum (complement source) were incubated at 37 °C for 50 min. Trypan blue dye was added during the last 7 min of incubation to stain dead sperm. Absorption was accomplished by reacting 0.14 ml of H-Y antiserum (diluted 1/2) with an equal volume of packed liver cells. The cells were suspended in the antiserum for 30 min and then discarded. Absorption in the cytotoxicity test is manifested as a decrease in cytotoxic titre, indicating that H-Y antibody has been removed specifically from a particular aliquot by the cells used in the absorption procedure and that these cells must therefore contain H-Y antigen.

marker to distinguish the *Sxr,XX* male from the XY and *Sxr,XY* classes. Two types of mating are used: in one, *Sxr,Ta/Y* males are crossed with $+/+$ females (F_1 hybrid, C3H $\times$ 101) and, in the other, *Sxr,+Y* males are crossed with *Ta/+* females from a stock also maintained on the C3H/101 background. XY and *Sxr,XY* males are distinguished on the basis of testis weight and their ability to produce *Sxr,XX* progeny.

We tested *Sxr,XX* mice for the presence of H-Y antigen by the three methods described for *Tfm/Y* mice³. (1) In

each of five separate experiments anti-H-Y serum gave a positive reaction in the cytotoxicity assay with dissociated epidermal cells from *Sxr,XX* males (Fig. 1a). In two of these experiments sibling XY males carrying *Sxr* (identifiable by small testes), and normal sibling XY males, were used as positive controls. In both experiments, one of which is shown in Fig. 1b, the anti-H-Y cytotoxicity reaction was stronger with *Sxr,XY* males than with normal XY males. This suggests the presence of excess H-Y antigen in *Sxr,XY* males (although this has not yet been corroborated by quantitative absorption of anti-H-Y serum) and thus might imply that *Sxr,XY* males have excess H-Y-determining genetic material. (2) The presence of H-Y antigen in *Sxr,XX* males was further indicated by a series of absorption tests; three of them, shown in Fig. 2, in which spleen, brain and liver cells of *Sxr,XX* males removed specific anti-H-Y reactivity from the H-Y antiserum. (3) C57BL/6 (B6) females grafted with *Sxr,XX* trunk skin were thereby specifically presensitized to give a second-set response when subsequently grafted with B6 male skin, indicating that the *Sxr,XX* skin graft was H-Y+ (Table 1).

These data support the interpretation that *Sxr* represents a cytologically undetected translocation between the Y chromosome and an autosome.

There are two possible mechanisms by which such a translocation may have arisen^{1,2} (Fig. 3). If *Sxr* arose as a simple chromosome-type rearrangement between an autosome and the Y chromosome, the original male presumably possessed a normal complement of chromosome material, although the Y chromosome itself would be deficient and an autosome would carry a segment of Y chromosome. According to this scheme his *Sxr,XX* offspring would obtain a male-determining portion of the Y chromosome; his XY sons would have a deficient but nevertheless male-determining Y chromosome, and his *Sxr,XY* sons should have the same chromosome complement that was attributed to their father. But this hypothesis does not explain the defective testicular development seen in the original male or in his *Sxr,XY* sons¹ (reviewed in ref. 2). Therefore the more likely origin of this translocation involves a rearrangement of the chromatid-type in the father of the original male, in which case the original male could have retained a normal Y chromosome plus a duplication of a region of the Y chromosome involved in the translocation¹. The defective testicular development of *Sxr,XY* males is understandable on the basis of this karyotype, since they would be partially equivalent to XYY males, which are known in the mouse to be subject to meiotic breakdown (reviewed in refs 2 and 6).

The interpretation of *Sxr* as a Y-autosome translocation may account for the different effect of *Sxr* on XX and XO genotypes. It has been proposed that the absence of germinal elements in XXY males arises because the presence of two active X chromosomes is incompatible with normal differentiation of male germ cells⁷. Early germ cell failure

Table 1 Second-set rejection of B6 male skin by B6 females previously grafted with *Sxr,XX* skin

First graft* (no. of B6 females grafted)	Rejection time (d) of B6 male skin grafted 2-3 months later								MST (confidence limits)
<i>Sxr,XX</i> (14)	10, 16,	11, 16,	12, 17,	15, 18,	15, 18,	16, 19,	16, 23		14.5 (16.3-12.9)
(C ₃ H $\times$ 101) F_1 XY male controls (14)	12, 15,	12, 16,	12, 17,	13, 17,	13, 17,	15, 21,	15, 22		14.5 (16.3-12.9)
(C ₃ H $\times$ 101) F_1 XX female controls (8)	14, 30	16, 30	21, 30	23, 30	23, 30	26, 30	28, 30		20.5 (17.2-24.4)

Summary of data for two experiments involving four *Sxr,XX* graft donors and three donors for each of the two control groups; these donors also provided spleen cells and tail-epidermal cells for serological tests (Figs 1 and 2). Trunk skin was grafted according to Billingham and Medawar⁸, using Band-Aids instead of plaster casts; rejection times were scored visually. Median survival time (MST) and confidence limits of the median were calculated by the method of Litchfield⁹.

*See Fig. 1.

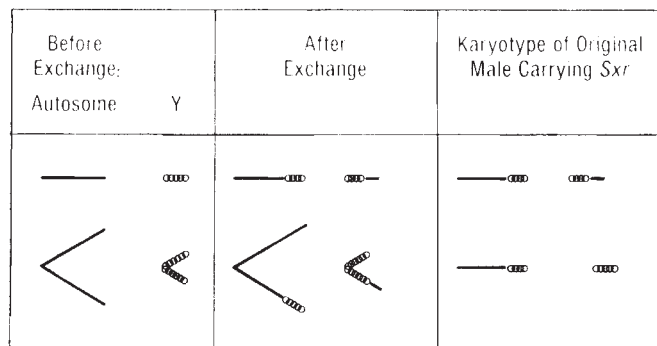


Fig. 3 Alternative hypotheses for translocational origin of Sxr. Upper, chromosome aberration; lower, chromatid aberration.

in Sxr,XX males and more extensive spermatogenesis in Sxr,XO males reinforces this argument. Furthermore, since spermiogenesis in Sxr,XO males is not completely normal, the translocated Y is presumably incomplete.

Although the data account for the male development of mice with an apparently female karyotype, different mechanisms must be responsible for the sex-reversed conditions found in goats and pigs (reviewed in refs 2 and 6). In these species the sex reversal is inherited as an autosomal recessive and this is not immediately understandable in terms of a Y autosome translocation. Clearly, the H-Y status of these sex-reversed individuals requires investigation.

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human, cultured somatic cells they yielded hybrid cells capable of indefinite multiplication^{8,9}. Applied to somatic cell protoplasts of two species of higher plants, they yielded hybrid protoplasts which regenerated, multiplied and developed into whole hybrid plants¹⁰. The list of cells and cell organelles fused has now expanded to include bacterial and fungal protoplasts and chloroplasts. It is reasonable to expect that PEG will induce the fusion of almost any two biological membranes and become an increasingly useful tool in their investigation^{6,11}.

We report here two features of PEG-induced fusion in cultured human fibroblasts. One is that fusion occurs preferentially between, or near to the tips of elongated processes. The other is that, contrary to previous interpretations^{8,9}, fusion occurs very near to, or even during, the time of PEG treatment.

The proportions of bi- and multinucleate cells at various times after pulse treatment with PEG of monolayers of early passage human skin fibroblasts were measured both by phase contrast microscopy^{8,9} and by fixing and staining. The time sequence is as follows: about one fifth of the final proportion of bi- and multinucleate cells are clearly identifiable as such within about 30 min at 37 °C after treatment this proportion goes on increasing over a number of hours (6 to more than 24) to reach a peak of 30–60% (Fig. 1). The gradual decline after this peak is an artefact not relevant to the present paper. However, two of the factors contributing to it were ascertained: (1) unfused cells multiply, while very few cells with three or more nuclei do so; (2) a proportion of binucleate cells undergo the reverse of fusion, that is, cell division without prior nuclear division. The proportion of binucleate cells which go on to fuse their nuclei is too small to contribute appreciably to the decline in question.

The gradual rise in the proportion of bi- and multinucleate cells over a number of hours after treatment was interpreted^{8,9} as indicating that fusion of treated cells could continue over many hours after removal of PEG. A first doubt on this interpretation arose from the following observation. In rather sparse fibroblast monolayers, where each cell sends out pseudopodia to touch other cells, configurations like those of Fig. 2 are commonly seen a few hours

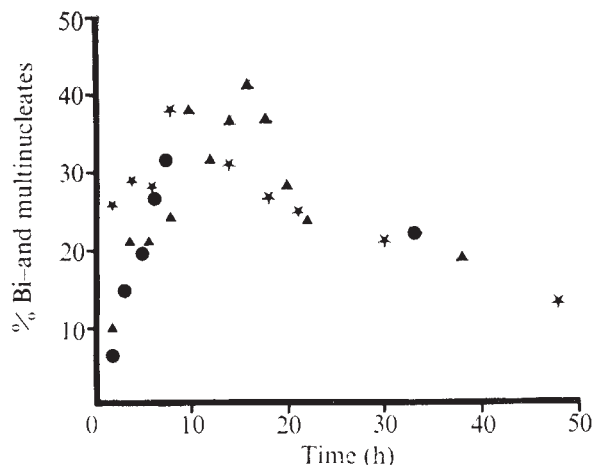


Fig. 1 Percentages of bi- and multinucleate cells at successive times after treatment of monolayers of human skin fibroblasts; PEG molecular weight 6,000, 50% (1w-1v) in Dulbecco's medium for 1 min followed by washes in three progressively more diluted solutions of PEG and finally growth in Dulbecco's medium with 10% foetal calf serum. Different symbols represent different experiments. A variety of other effective treatments of monolayers with PEGs of different molecular weights, at different concentrations and with or without addition of dimethylsulphoxide¹² all show the same general trend: an initial increase with time of the proportions of bi- and multinucleate cells followed by a decline. For the meaning of this decline after the peak see text.

Time and mode of fusion of human fibroblasts treated with polyethylene glycol (PEG)

SOMATIC cell fusion has become a powerful tool in cell biology and genetics¹⁻³. Until recently the standard reagent for increasing the rate of fusion in cultures of animal cells was inactivated Sendai virus⁴. In 1974 polymers of ethylene glycol (polyethylene glycol, PEG), over a wide range of molecular weights, were shown to be very effective, non-toxic chemical 'fusogens' for higher plant protoplasts⁵. They were then applied to fuse hen erythrocytes⁶ and these with yeast protoplasts⁷. Applied to mammalian, including

after treatment and before the peak proportion of bi- and multinucleate cells is reached. Points of contact between cells before treatment often involve a pair of thin elongated pseudopodia, one from each of two cells, or one pseudopodium from one cell in contact with the main body of another cell. Those pseudopodia which have fused following treatment later increase in diameter and contract in length. It takes time for two cells so connected to coalesce through an initially thin long connection. Two cells in the early stages of such coalescence would not be classified as a single binucleate cell. This mode of fusion is reminiscent of the fusion of myoblasts to form myocytes.

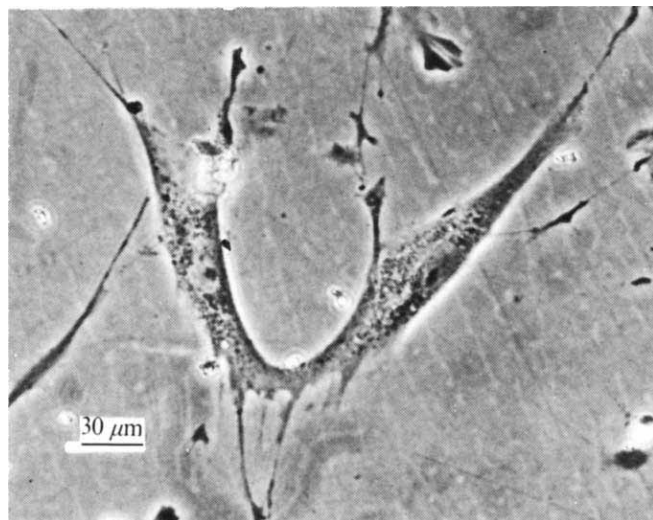


Fig. 2 Monolayer of human skin fibroblasts treated with PEG as in Fig. 1: a commonly seen configuration resulting from two cells which at the time of treatment fused at or near the tips of elongated filaments and gradually coalesced their cytoplasms. Phase contrast photography 3 h after treatment.

Time-lapse phase cinemicrophotography has provided an unequivocal answer. Applied to human fibroblasts in monolayer, treated for 1 min with 50% PEG (molecular weight 6,000), it has revealed three facts. First, some bi- and multinucleate cells are already classifiable as such soon after treatment. Second, apparently delayed 'fusions' are of the type just mentioned: two cells connected at the end of long thin pseudopodia coalesce their cytoplasms by slow contraction of these pseudopodia which fused near their tips at, or soon after, the time of treatment. The nuclei of such pairs of cells eventually come to lie next to each other in what has become a common cytoplasm, but this may take many hours. Third, time-lapse recording over more than 24 h of all the cells in individual fields has shown many cases of two cells initially far apart moving to touch each other. In no case, however, has fusion followed. If the increase with time of the proportion of bi- and multinucleate cells in monolayers were due to extensive fusion of such cells after removal of PEG we would have observed several such fusions.

Another kind of evidence comes from work on the time sequences of appearance of bi- and multinucleate cells in treated monolayers as compared with treated cell suspensions. The procedure developed by us and routinely used for treating cell suspensions¹² is to take up gently a well drained pellet of some 10^6 cells in 0.5 ml of PEG molecular weight 1,000 1w+1.4v in Dulbecco's modified medium with 15% dimethylsulphoxide¹³, dilute this suspension gently within 1 min with an equal volume of PEG molecular weight 1,000 1w+3v without dimethylsulphoxide, and again with 4 ml of medium. This final suspension is then dispensed in flasks or dishes containing a volume of medium with serum at least 10 times greater. In contrast to treated monolayers (Fig. 1), the proportion of bi- and multinucleate cells classi-

fiable as such from cells treated in suspension does not show the gradual rise in the first 24 h. The long time required by the cytoplasms of cells in monolayer to coalesce through thin long connections is not required by the rounded up cells of a suspension. It is important now to ascertain whether or not also in these cells fusion occurs prevalently between the tips of microvilli, or other microprocesses and, in general, between regions of high curvature.

Thus, as to the time of fusion, we conclude that whether two or more cells will ultimately coalesce and become recognisable as a single bi- or multinucleate cell is determined during or very near the time of treatment. In treated monolayers this final coalescence may take many hours, whereas in treated suspensions it occurs and can be recognised soon after treatment.

As to the mode of fusion, we conclude that in sparse monolayers it occurs mainly at or near the tips of filamentous processes of which every cell in low density cultures has many. This explains why the proportions of bi- and multinucleate cells found in treated sparse monolayers are as high as, or even higher than in confluent ones. In the latter, there is very extensive side-by-side contact between fibroblasts. Yet this does not lead to more fusion. It will be important to compare some of the physicochemical properties of the cell surface, such as charges, or distribution of intrinsic proteins, near the tips of processes with those on the main cell body (see for example refs 11 and 14). This preferential fusion near the tips of processes may well underlie the observation of a negative correlation between density of cells and proportion of mononucleate hybrids arising from PEG-treated mixed monolayers. This correlation was attributed to the lesser formation of large multinucleate cells in sparse monolayers. Instead it may well be the consequence, in part at least, of a greater fusion efficiency per cell in sparse monolayers due to the greater number of filamentous processes that fibroblasts show under this condition.

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Loss of duplicate gene expression after polyploidisation

INCREASES in cellular DNA content have been important in the evolution of eukaryotes¹⁻³. One mechanism for increasing DNA content is polyploidisation, which has been observed in a wide variety of organisms⁴⁻⁸, and which is believed to have preceded the evolution of the early vertebrates⁹. It has been suggested that, following gene duplication, many of the redundant copies would be silenced early

in evolution by mutation^{10,11}. Those duplicate genes remaining expressed are then available to diverge in structure and acquire new functions⁹. We present evidence of extensive gene silencing in the 50-Myr history of a family of tetraploid fishes. Furthermore, those species regarded by systematists as advanced (that is, morphologically divergent from the ancestral form) may have lost the expression of more duplicate genes than primitive species (that is, those with a morphology similar to the ancestral form).

Theoretically, the most useful group of organisms for the analysis of gene loss after polyploidisation would be one which has undergone polyploidisation relatively recently (before complete diploidisation has taken place), yet not so recently that the enzymes encoded by the duplicate genes are still indistinguishable. The Catostomidae meet these requirements and thus provide an excellent model system for investigating genome evolution after polyploidisation. Since their origin 50 Myr ago¹², sufficient time has elapsed for many changes at the levels of morphology and structural genes. At the chromosome level, however, all genera have conserved a tetraploid karyotype with $2n=100$, compared with $2n=50$ for most cypriniformes¹². In addition, the DNA content of catostomid species examined is twice that of typical diploid fishes¹². On the basis of morphology these catostomids have been placed in three subfamilies. Two primitive subfamilies (Cycleptinae and Ictiobinae) closely resemble fossil forms (*Amyzon*, Miocene) in morphology¹³⁻¹⁵. A single advanced subfamily (Catostominae) has diverged considerably in morphology. Starch gel electrophoresis of 20 enzymes was employed to reveal the extent of duplicate gene expression. The application of Nei and Chakraborty's formula¹⁶ for the probability of electrophoretic identity of two proteins, with a time span of 50×10^6 yr, allows us to estimate conservatively that the isozymes encoded by duplicate genes will be separable by electrophoresis at least 95% of the time.

The distributions of duplicate genes expressed are presented in Tables 1 and 2. Considerable extinction of gene expression has occurred. For the entire family, the average loss of duplicate gene function is 53% (range 35% to 65%). The average percentage is similar to that observed in the few species of salmonid¹⁷ and cyprinid¹⁸ fish which have been studied. In many cases where two catostomid species have the same percentage of duplications expressed, different combinations of loci have undergone the diploidisation

process. There is no obvious correlation with the metabolic role of an enzyme and the extent to which the duplicate loci encoding it have undergone diploidisation.

Several lines of evidence argue against the genes being silenced randomly throughout the entire 50-Myr period. It is commonly assumed that one of the duplicate loci is free to accumulate what would have been previously 'forbidden' mutations as long as the other locus remains functional. Can the drift to fixation of non-functional (null) alleles over 50 Myr account for the amount of diploidisation observed in the catostomids? It has been shown¹¹ that the probability, P , of a null allele drifting to fixation at a duplicate locus is given by

$$P = 1 - [4Nu + 1][\exp(-ut)] \quad (1)$$

If we assume a typical mutation rate $u = 1 \times 10^{-6}$ per peptide per generation^{19,20}, the number of generations, $t = 25 \times 10^6$, and a 'generous' estimate of an effective population size for a typical catostomid species, $N = 10^5$, then it is calculated that $P > 0.9999$. On the basis of this theory most duplicate loci should have been lost after a 50-Myr period, a prediction which is in disagreement with our observation that only 53% (on the average) of the duplicate loci have been lost. Although null alleles may have drifted to fixation shortly after polyploidisation, it is unlikely that they are currently doing so. The fact that polymorphisms for null alleles have not been observed in the catostomids suggests that fixation occurs rapidly. An additional consideration is that many of the duplicate genes have diverged in their tissue and developmental specificity and are differentially regulated^{21,22}. We have observed many instances of such differential regulation. The duplicate Ldh-B loci in *Catostomus commersoni*, for example, are equally expressed in mature gonads, while only one locus is expressed in most other tissues. Because some of the duplicate loci have diverged in function and regulation, and because we have observed levels of duplicate gene expression much higher than predicted by random loss, it is likely that these duplicate genes are at present being either maintained or quenched by selection.

An evolutionary trend of diploidisation can best be seen by investigation of a large number of genera. One important inference generated by the present study is that species of the primitive subfamilies have retained more functionally duplicate genes than those of the advanced subfamily. An

Table 1 Number of duplicate genes expressed in the primitive catostomids

Species	N*	Enzyme locus																	% Gene duplications expressed			
		Ck-B	Sod	M-Mdh	Gpi-A	Gpi-B	G3Pdh	Ak-A	6Pgd	Ldh-B	S-Aat	Ald-C	Acp	Ck-A	Mdh-A	Mdh-B	Ldh-A	Ldh-C		Adh	Pgm	Xdh
Cycleptinae																						
<i>Cycleptus elongatus</i>	(15)	2	2	2	2	2	2	2	1	2	1	1	2	2	2	2	2	1	1	1	1	65
Ictiobinae																						
<i>Ictiobus bubalus</i>	(30)	2	2	2	2	2	2	2	2	1	2	2	2	2	1	1	1	1	1	1	1	60
<i>I. niger</i>	(6)	2	2	2	2	2	2	2	2	1	2	2	2	2	1	1	1	1	1	1	1	60
<i>I. cyprinellus</i>	(25)	2	2	2	2	2	2	2	2	1	2	2	2	2	1	1	1	1	1	1	1	60
<i>Carpiodes carpio</i>	(25)	2	2	1	2	2	2	2	2	2	2	2	1	1	1	2	1	1	1	1	1	55
<i>C. velifer</i>	(20)	2	2	1	2	2	2	2	2	2	2	2	1	2	1	2	1	1	1	1	1	60
<i>C. cyprinus</i>	(25)	2	2	1	2	2	2	2	2	2	2	2	1	1	1	2	1	1	1	1	1	55
Average % of duplicate genes expressed = 59																						

The number of duplicate genes expressed for specific enzymes 50 Myr after tetraploidisation. The percentage of gene duplications still expressed is given for individual species of primitive catostomids. 2 indicates the presence of duplicate loci for an enzyme, 1 designates that only one locus is expressed. N* is the exact number of individuals examined for each locus for most species; however, for a few species, it represents an average among the loci studied. Late juvenile to adult fish were stored frozen, homogenised 1:2 (v/v) in Tris-HCl, pH 7, at 4 °C centrifuged 27,000g for 20 min, and the supernatant electrophoresed. Ak (adenylate kinase), Aat (aspartate amino transferase), Acp (acid phosphatases), Mdh (malate dehydrogenase-NAD), Pgm (phosphoglucotomutase), and G3Pdh (glycerol-3-phosphate dehydrogenase-NAD) were resolved on histidine-NaOH buffer²³; 6Pgd (6-phosphogluconate dehydrogenase-NADP), Ldh (lactate dehydrogenase-NAD), Ald (aldolase), and Sod (superoxide dismutase) on Tris-citrate²⁴; Ck (creatine kinase), Gpi (glucose phosphate isomerase), and Sod on Tris-EDTA-borate²⁵; Adh (alcohol dehydrogenase-NAD), and Xdh (xanthine dehydrogenase-NAD) on borate-NaOH²⁶. Enzyme staining modified from Shaw and Prasad²⁷. For most loci, various tissues were analysed. A diploid control, *Notropis stramineus*, showed no evidence of gene duplication.

Table 2 Number of duplicate genes expressed in the advanced catostomids

Species	Enzyme locus																				% Gene duplications expressed
	N	Ck-B	Sod	M-Mdh	Gpi-A	Gpi-B	G3pdh	Ak-A	6Pgd	Ldh-B	S-aat	Ald-C	Acp	Ck-A	Mdh-A	Mdh-B	Ldh-A	Ldh-C	Adh	Pgm	
Catostominae																					
<i>Erimyzon sucetta</i>	(22)	2	2	2	1	2	1	2	2	2	2	1	1	1	1	2	1	1	1	1	45
<i>E. oblongus</i>	(25)	2	2	2	1	2	1	2	2	2	2	1	1	1	1	2	1	1	1	1	45
<i>Minytrema melanops</i>	(25)	2	2	2	2	2	1	1	1	1	1	2	1	1	1	2	1	1	1	1	35
<i>Moxostoma erythrurum</i>	(35)	2	2	2	2	1	1	2	1	1	2	1	1	1	2	1	1	1	1	1	35
<i>M. duquesnei</i>	(30)	2	2	2	2	1	1	2	1	2	1	1	1	1	2	1	1	1	1	1	35
<i>M. macrolepidotum</i>	(35)	2	2	2	2	1	1	2	2	1	1	1	1	1	2	1	1	1	1	1	35
<i>M. cervinum</i>	(35)	2	2	2	2	1	2	2	2	2	2	1	1	1	2	1	1	1	1	1	50
<i>M. rhotocum</i>	(12)	2	2	2	2	1	2	1	2	1	1	1	1	1	2	1	1	1	1	1	35
<i>Hypentelium nigricans</i>	(30)	2	2	2	2	1	2	1	2	1	1	1	1	1	2	1	1	1	1	1	35
<i>H. etowanum</i>	(10)	2	2	2	2	1	2	1	2	1	1	1	1	1	2	1	1	1	1	1	35
<i>Catostomus commersoni</i>	(35)	2	2	2	2	2	2	2	2	2	1	1	1	1	2	1	1	1	1	1	50
<i>C. catostomus</i>	(17)	2	2	2	2	2	1	2	2	2	1	1	1	1	2	1	1	1	1	1	45
<i>C. occidentalis</i>	(11)	2	2	2	2	2	1	2	2	2	1	1	1	1	2	1	1	1	1	1	45
<i>C. macrocheilus</i>	(5)	2	2	2	2	1	1	2	2	1	1	1	1	1	2	1	1	1	1	1	40
<i>C. columbianus</i>	(14)	2	2	2	2	2	1	2	2	2	1	1	1	1	2	1	1	1	1	1	45
<i>C. luxatus</i>	(2)	2	2	2	2	2	1	2	2	2	1	1	1	1	2	1	1	1	1	1	45
<i>C. plebeius</i>	(25)	2	2	2	2	1	1	2	2	2	1	1	1	1	2	1	1	1	1	1	40
<i>Chasmistes brevirostris</i>	(1)	2	2	2	2	2	1	2	2	2	1	1	1	1	2	1	1	1	1	1	45
<i>Xyrauchen texanus</i>	(11)	2	2	2	2	2	2	2	2	2	1	1	1	1	2	1	1	1	1	1	50
Average % of gene duplications expressed															42						

The number of duplicate genes expressed for specific enzymes 50 Myr after tetraploidisation. The percentage of gene duplications still expressed is given for individual species of advanced catostomids. 2 indicates the presence of duplicate loci for an enzyme, 1 designates that only one locus is expressed. Abbreviations and methods are as explained in Table 1.

average of 59% of the loci show evidence of duplication in the primitives (12/20) compared to 42% in the advanced (8/20). Within the advanced subfamily, relatively primitive species such as *Moxostoma cervinum*²² and *C. commersoni*²³ have more functionally duplicate genes than highly morphologically divergent species such as *M. rhotocum* and *C. plebeius* (see Table 2). If the quenching of genes were completely random through time, or if tandem duplications occurred to any extent, the above relationship would not be evident. A phylogeny has been constructed, based on the comparison of the presence of one or two functioning gene loci, which yields a 'tree' similar in many respects with those based on morphology. From these considerations we conclude that species which are conservative at the morphological level are also conservative with respect to the retention of duplicate gene expression. In an analysis parallel to our study, Hinegardner²⁴ has found that the more generalised fish orders have higher cellular DNA contents than highly specialised orders. Our working hypothesis is that phenotypically advanced organisms lose more genetic information. After an initial DNA increase by polyploidy, the first stage of evolution will be a moderately rapid loss of the expression of redundant genes, as found in the catostomids. Then, over a longer period, this DNA which is not expressed is physically eliminated²⁴. The catostomids are probably still in the first stage in view of their high DNA content.

In summary, during 50 Myr of post-polyploid evolution, the catostomids have returned to a functionally diploid state at over half the enzyme loci examined. This process is not entirely random. We suggest that some duplicate genes have been quenched more rapidly in the morphologically advanced species than in the morphologically conservative species.

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Common precursor pathways of Herpes DNA and of repair synthesis in ultraviolet irradiated cells

DEOXYRIBONUCLEOTIDE precursors of DNA synthesis may be supplied by two alternative pathways in mammalian cells¹: (1) endogenous synthesis by reduction of the corresponding ribonucleotides at the diphosphate level, except thymidine nucleotides which are produced by deamination and methylation of cytidine deoxynucleotides at the monophosphate level; (2) exogenous salvage synthesis by stepwise phosphorylation of pre-existing deoxyribonucleosides. The first step leading to monophosphates is catalysed by purine² and pyrimidine^{3,4} deoxyribonucleoside kinases. In cells infected with Herpes simplex type I (HSV) there is an induction of both thymidine kinase (TK)⁵ and deoxycytidine kinase activity⁶. These two activities seem to be a property of the same virus-specified enzyme⁷, whose role

Table 1 Effects of inhibitors of endogenous pathways on HSV production by ultraviolet-irradiated and non-irradiated stationary CV-1 cells

Treatment	Dose of ultraviolet (erg mm ⁻²)			
	0	120	240	480
	Virus yield as % of the corresponding non-treated cultures			
None	100 (1.3 × 10 ⁸ †)	100 (3.1 × 10 ⁸ †)	100 (9.1 × 10 ⁷ †)	100 (2.3 × 10 ⁷ †)
Hydroxyurea, 10 mM	0.8–1.3‡	3.6	3.0	3.3–4.5‡
Cytosine arabinoside, 0.1 mM	0.2–0.4	0.65	1.4	2.7–3.5
Fluorodeoxyuridine, 2 mM	7–12	22	45	67–87
FUdR, 2 mM + thymidine 2 mM	64–71	—	—	187–207
Thymidine, 2 mM	55–70	—	—	123–144

Stationary CV-1 cultures (2 × 10⁵ cells cm⁻²) were kept before irradiation and infection with HSV in the presence of a minimum essential medium (MEM) without serum and tryptose phosphate broth, as previously described¹⁰. HSV was grown in the presence of MEM. The viral yield by control cultures was between 150 and 350 PFU per cell.

*The drugs were present during the 18 h of the viral cycle.

†PFU per Petri dish in the non-treated cultures.

‡Extreme values of three separate experiments.

remains unclear. It seems to be important for viral DNA synthesis in resting cells⁸ in which the endogenous metabolism of DNA is low whereas the degradative phosphatase activity is high⁹. On the other hand viral TK activity increased faster during the exponential phase of viral DNA synthesis in cells irradiated with ultraviolet light before infection than in non-irradiated cells¹⁰. This suggested that viral TK may have a more important role in the synthesis of HSV DNA in ultraviolet-irradiated than in non-irradiated cells. I present here results showing that the exogenous pathways are preferentially used for both HSV replication and for DNA repair synthesis in ultraviolet-irradiated cells.

All experiments were performed in stationary (2 × 10⁵ cells cm⁻²) monkey kidney CV-1 cells. Culture, irradiation and infection of cells and the measurements of single cycle virus yield were carried out as previously described¹⁰.

First, I measured the production of infectious virus by irradiated and control cultures in the presence of inhibitors of endogenous pathways such as fluorodeoxyuridine (FUdR) and cytosine arabinoside (ara-Cdr) which block the synthesis of deoxythymidilate¹¹ and of deoxycytidilate¹², respectively, and hydroxyurea (HU) which mainly inhibits the reduction of ribo- to deoxyribonucleotides^{13,14}. Table 1 shows that viral production was more inhibited by these drugs in control than in irradiated cultures. The extent of inhibition of viral production by FUdR and ara-Cdr decreased as the dose of ultraviolet increased. Since both drugs must be phosphorylated in order to inhibit DNA synthesis^{11,15,16}, this effect could be due to a lower rate of phosphorylation of these inhibitors as a consequence of ultraviolet irradiation. I found that FUdR and deoxycytidine were phosphorylated to a lesser extent in extracts from ultraviolet-irradiated and infected

cells than in extracts from controls (data not shown). HSV production was no longer impaired in the presence of both thymidine and FUdR (2 mM each) in non-irradiated cultures (Table 1). This might indicate that the exogenous supply of thymidine nucleotides is sufficient under these conditions to overcome the corresponding endogenous block induced by FUdR. However, one cannot exclude the possibility that viral and (or) cell TK have greater affinity for thymidine than for FUdR, leading to a lower rate of phosphorylation of the latter drug. In addition, Table 1 shows that in the presence of thymidine alone (2 mM) HSV production decreased in non-irradiated cultures and increased in irradiated cultures.

I measured in parallel the amount of incorporation of the four deoxyribonucleosides into DNA of uninfected cells in the presence of these inhibitors. This incorporation was more inhibited in control than in irradiated cultures (Table 2). The pool sizes of the free deoxyribonucleosides are unknown but probably differ in the two conditions since they depend on the rate of formation and of disappearance of deoxyribonucleosides which are both altered after irradiation as a result of the increase in nuclease activities leading to free deoxyribonucleosides¹⁷ and by the depression of the rate of DNA synthesis¹⁸. As the deoxyribonucleoside concentrations used here (3 × 10⁻⁷ M) are sufficient to modify the natural pool size of the free deoxyribonucleosides occurring in normal cultures¹⁹, it is not possible to obtain quantitative data from these last results. It can only be concluded that inhibitors of endogenous DNA synthesis are less effective (1) on the replication of HSV in ultraviolet-irradiated cells than in control cultures, (2) on the incorporation of the four deoxyribonucleosides into cellular DNA during post-ultraviolet DNA synthesis than during normal synthesis.

Table 2 Incorporation of deoxyribonucleosides into the DNA of normally growing and stationary ultraviolet-irradiated CV-1 cells in the presence of inhibitors of endogenous pathways

Treatment	Incorporation as % of controls of:							
	Deoxyadenosine		Deoxycytidine		Deoxyguanosine		Thymidine	
	A*	B*	A	B	A	B	A	B
None	100	100	100	100	100	100	100	100
	(9,975†)	(1,850†)	(3,360)	(1,060)	(10,810)	(2,620)	(19,320)	(3,990)
Fluorodeoxyuridine, 1 mM	48	86	32.6	49.5	46	60	43	117
Cytosine arabinoside, 0.1 mM	20.2	50	12.3	35	24.2	36	7	30
Hydroxyurea, 10 mM	15.1	55	33	360	12.7	51.5	4.9	30.6

CV-1 cells were grown in 3 cm diameter Petri dishes, then kept in the presence of each of the ³H-deoxyribonucleosides at 2 µCi ml⁻¹ + the four unlabelled deoxyribonucleosides to obtain a final concentration of 0.3 nmol ml⁻¹ each, in MEM without serum (stationary) or with 5% dialysed newborn calf serum (growing). After 18 h, cells were rinsed three times with PBS, scraped into PBS, ultrasonicated (2 min at 4 °C), treated with DNase-free pancreatic RNase (20 µg ml⁻¹, 3 h at 37 °C) to digest the labelled RNA, then the 5% TCA-insoluble radioactivity was precipitated on to GF/C filters and counted as described¹⁰. The given values are the mean values of three Petri dishes.

*A, growing (3 × 10⁴ cells cm⁻²) cultures.

B, stationary ultraviolet-irradiated (240 erg mm⁻²) cultures.

†Actual values for c.p.m. per Petri dish in the controls (mean values of three dishes).

The mechanism by which inhibitors of endogenous pathways decrease the incorporation of labelled deoxyribonucleosides by the exogenous pathways during normal DNA synthesis remains unknown. The cyclic increase of the TK activity at the beginning of the S phase¹⁹ strongly suggests that the corresponding exogenous pathway has an important role in normal DNA synthesis, possibly as a salvage pathway²⁰. An interrelated use of both endogenous and exogenous pathways could explain the mechanism mentioned above. In contrast, the excision-repair synthesis which occurs in these stationary ultraviolet irradiated CV-1 cells in the absence of normal DNA synthesis¹⁰ would necessitate a low concentration of deoxyribonucleosides. This could be achieved by the exogenous pathways which are economical since (1) they permit the re-utilisation of the breakdown products arising from the irradiated DNA as previously shown¹⁰, (2) they require only phosphorylating enzymes compared with the numerous enzymes involved in the endogenous pathways.

To test this last possibility I measured the level of the four deoxyribonucleoside kinases in extracts of stationary cultures made at different times after irradiation during the 48-h period¹⁰, and found that the deoxyribonucleoside kinase activities decreased slightly by 6 h after irradiation, then increased, attaining a value at 24 h which was greater than that found prior to irradiation (Table 3).

Table 3 Phosphorylating activities of extracts of stationary CV-1 cells at different times after ultraviolet irradiation

³ H-precursor	Time (h) after irradiation with 240 erg mm ⁻²				
	0	6	12	24	48
Deoxycytidine	100 (1†)	62*	111*	140*	106*
Deoxyguanosine	100 (0.98)	77	122	128	110
Thymidine	100 (2.41)	85	119	141	126

Stationary cultures were kept in MFM without calf serum and tryptose phosphate broth between irradiation and extraction. Cell extracts were made and their enzyme content determined as described by Lin and Munyon²¹. For each cell extract at least three dilutions were tested over a range of protein concentrations where the reaction was linear (500–3,000 µg ml⁻¹). The final concentrations of the non-radioactive deoxyribonucleosides in the mixture reactions were, for deoxyadenosine and deoxyguanosine: 4×10^{-6} M; for deoxycytidine and thymidine: 2×10^{-5} M.

*The values are given as the percentage of the values obtained at time 0.

†nmol of deoxyribonucleoside phosphorylated per mg protein in 30 min at 38 °C.

The activity of deoxyadenosine kinase was too low to be detected in these conditions. The variations of the deoxyribonucleoside kinase activities observed after ultraviolet irradiation seem not to be due to the occurrence of any normal DNA synthesis, since similar results (unpublished observation) were obtained in extracts from cells kept in the presence of 2 mM of each of the four deoxyribonucleosides after irradiation, that is, in conditions where normal DNA synthesis must be inhibited by an excess of non-radioactive deoxyribonucleosides^{22,23}. The increase of these kinase activities starting 6 h after irradiation could reflect either the ultraviolet-induced synthesis of new enzyme molecules, or the alterations of the activities of pre-existing enzymes, for example by small effector molecules like dTTP¹⁹ leading to an end-product inhibition. Moreover, these results suggest a role of the deoxyribonucleoside kinases in DNA repair synthesis since their activities attain a maximum level at about the same time (24 h) at which DNA template function is fully restored, as shown by the recovery of interferon mRNA production²⁴ and of 45S ribosomal RNA transcription²⁵ in ultraviolet-irradiated stationary CV-1 cells.

In conclusion, it seems that deoxyribonucleoside precursors of DNA synthesis are supplied essentially by the exogenous pathways in post-ultraviolet DNA synthesis whereas in non-irradiated cells both exogenous and endogenous pathways are important in CV-1 cells. A similar pattern seems to occur for DNA synthesis of HSV grown in ultraviolet CV-1 cells as compared with virus grown in control cells. This conclusion is drawn from our earlier results¹⁰ and from those obtained with inhibitors of endogenous pathways of cell and of viral^{26,27} DNA synthesis. Such a pattern could explain that in the presence of thymidine at high concentrations, HSV production is slightly impaired in control cultures and increased in irradiated cultures (Table 1).

These results would indicate that the metabolic pathways are common to host cell and to HSV DNA synthesis. In this case, there might be a correlation between the residual ability to support HSV replication and the extent of repair synthesis in different ultraviolet-irradiated cell lines. I am currently investigating this possibility using normal skin cells and xeroderma pigmentosum cell lines which are defective in ultraviolet-induced repair synthesis.

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Maternal histidine metabolism and its effect on foetal development in the mouse

THERE have been reports^{1,2} of possible deleterious effects on the mental development of children due to maternal histidinaemia, in which a deficiency of the enzyme histidase leads to the exposure of the foetus to abnormally high concentrations of histidine and its imidazole derivatives. This is analogous to the well established case of maternal phenylketonuria³ which can lead to mental retardation in metabolically normal children. There is also a suggested link⁴ between low maternal concentrations of two enzymes in galactose metabolism and infantile cataracts. We report

Table 1 Plasma amino acid values for different genotypes on various diets

Diet	Genotypes	Genetic background
	<i>his/his</i> <i>his/+</i> <i>+/+</i>	
Normal	2.83 ± 0.18	50% Peru/50% C57
Normal + 2% His	6.08 ± 0.35	
Normal + 8% His	—	
'Low histidine'	0.44 ± 0.15	
Normal	2.97 ± 0.50	Peru
Phenylalanine		
Normal	—	C57
Normal + 10% Phe	—	
Lysine		
Normal	—	
Normal + 10% Lys	—	
Proline		
Normal	1.92 ± 0.082	50% PRO:RE/50% C57
Normal + 2%	4.18 ± 0.87	

The normal diet was FFG/LAC (Fox Foods) ground into a powder and fed as damp mash *ad libitum* at 1700 h each day with or without additives (% by weight), histidine-HCl, phenylalanine, lysine, proline (Sigma). Because of the diurnal variation in feeding, the plasma values shown are not present during the whole 24 h. Blood was taken between 0900 and 1000 h. We have evidence that higher levels are present in the early morning and that by 1700 h the levels can drop to about 50%. The low histidine diet was a mixture of 75% histidine-free diet (HF-2, Cow and Gate) 25% normal diet plus 2% vitamin supplement (Nutritional Biochemicals). All determinations of amino acids were carried out by ion exchange column chromatography (ninhydrin) with α -guanidopropionic acid as an 'external' standard added before deproteinisation by sulphosalicylic acid. All are expressed as $\mu\text{mol ml}^{-1}$ with their standard errors.

here work on the nature of the maternal effects of histidinaemia using mutant mice which are the homologues of the human condition⁵⁻⁷.

The activity of histidase is very low in the mouse mutant homozygotes (*his/his* < 5% activity of *+/+*) resulting in up to 20 times the normal level of histidine and its derivatives in all tissues. Heterozygotes, with about 50% enzyme activity, have normal histidine levels on normal diets^{5,6}. The histidinaemic mice have no overt abnormalities. On the other hand, some offspring of histidinaemic mothers, even when such offspring are metabolically normal, (*his/+*), display a balance defect resulting in circling behaviour which is associated with some or all of the following: deafness, damage during development *in utero*^{5,6} to the canals, cochlea and otoliths of the inner ear.

We have now found that this maternal effect can also be produced by high histidine diet of pregnant mothers who, on normal diet, would give birth to normal offspring. We have also established that the middle third of pregnancy is the critical period. Conversely, it is possible to reduce the incidence of abnormal offspring of genetically histidinaemic

mothers by reducing the histidine content of their diet during the second week of pregnancy. The exposure of the foetus to abnormal metabolic conditions of the mother, is, however, not the only determining factor for this condition. It seems that the genetic background can play an important role in influencing the susceptibility of the foetus to the metabolically generated teratogen. The murine maternal histidinaemia discussed here may well be of relevance to the aetiology of the homologous human abnormalities.

We were able to induce histidinaemia by purely dietary means. When heterozygous mice (*his/×*) are placed on an 8% histidine-supplemented diet the levels approach those of histidinaemic mice; homozygous wild-type mice (*+/+*) on this diet have only slightly higher levels than normal and were not used (Table 1). When we fed this diet to heterozygotes during the second week of pregnancy 14 balance defective offspring were produced out of 130 in 21 litters, compared with none in 209 animals on unsupplemented diet. No abnormal offspring were obtained from mothers fed the special diet during the first or third week. The critical period parallels that for *in utero* damage of the ear

Table 2 Effect of an 8% histidine-supplemented diet during the second week of pregnancy on the offspring of heterozygous (*his/+*) mothers

Diet	No. of offspring	Offspring genotypes	Effect on		Effect on inner ear			
			Balance	Hearing	Canals	Left Otoliths	Right Canals	Otoliths
(a) Offspring of mothers on 8% supplement	3	1 <i>his/his</i> ; 2 <i>his/+</i>	Severe	Severe	Abnormal	Missing	Abnormal	Missing
	1	<i>his/his</i>	Mild	Mild	Normal	Missing	—	—
	1	<i>his/+</i>	Mild	Severe	Abnormal	Missing	Normal	Missing
	1	<i>his/his</i>	Normal	Mild	Normal	Missing	Abnormal	Missing
	2	2 <i>his/+</i>	Normal	Mild	Normal	Missing	Normal	Missing
	1	<i>his/+</i>	Mild	Normal	Abnormal	Altered	Abnormal	Altered
	2	2 <i>his/+</i>	Normal	Normal	Abnormal	Missing	Abnormal	Missing
	12	8 <i>his/his</i> ; 4 <i>his/+</i>	Normal	Normal	Normal	Missing	Normal	Missing
	11	5 <i>his/his</i> ; 6 <i>his/+</i>	Normal	Normal	Normal	Normal	Normal	Normal
	19	11 <i>his/his</i> ; 8 <i>his/+</i>	Normal	Normal	Normal	Normal	Normal	Normal
(b) Offspring of untreated mothers								

The mice used were of 50% Peru/50% C57BL background. The treated and untreated animals were contemporaries so that variation in penetrance of the balance defect due to relaxation of selection (see text) was not relevant. The heterozygous (*his/+*) mothers were mated to homozygous (*his/his*) fathers so that the offspring listed above were 50% *his/his*, 50% *his/+*. Some of each genotype were affected. Balance was tested by spinning the animal by the tail and observing in a perspex cage. The animal was severely affected if it circled and mildly if it leant its head on one side^{1,2}. Hearing was tested with a short, sharp high pitched noise when the animal was at rest. A severe effect was no reaction of the ears or body any time the animal was tested and mild was no reaction 50% of the time. It can be seen that an effect on balance is only observed when both canals and otoliths are affected.

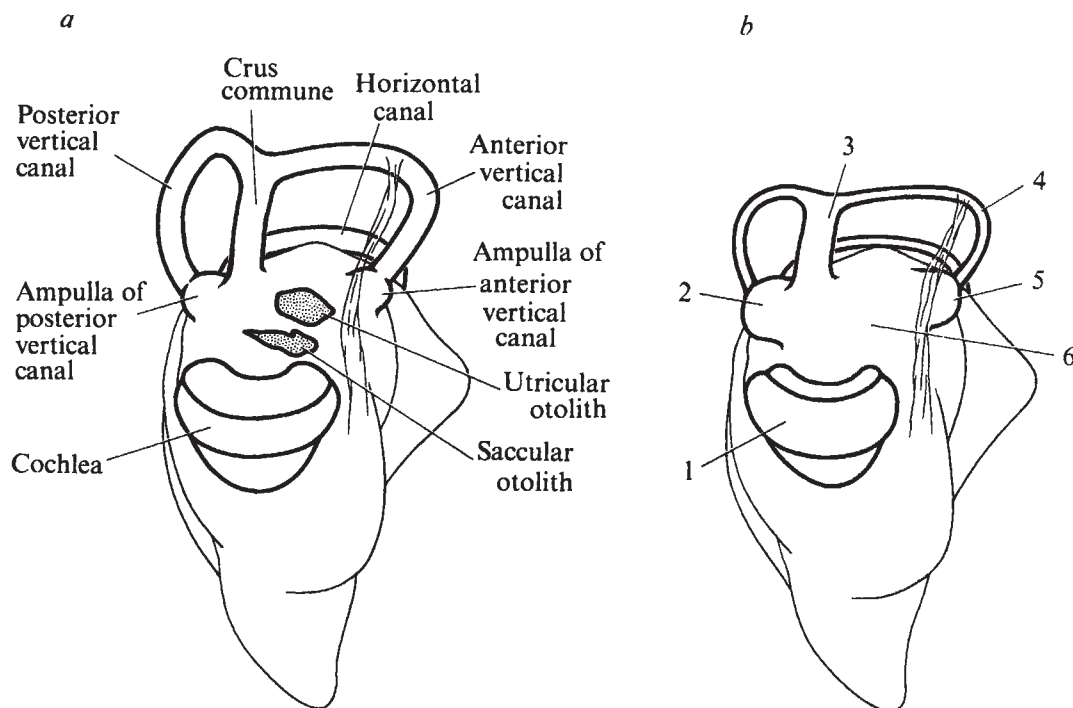


Fig. 1 Diagram of the abnormalities seen in the whole mounts of adult ears. *a*, Normal; *b*, affected. The ears were dissected into 75% alcohol and eventually cleared in benzyl alcohol. They were examined under the normal light and polarised light microscope. Canals are considered abnormal if they are shortened, thin or misshapen; otoliths are altered if they are enlarged or thin. About half the offspring from both treated and untreated mothers have utricular or saccular otoliths displaced but not damaged. This occurs in animals of the Peru or hybrid Peru/C57BL strains but not with pure C57BL. Any one ear may show one or more of the following abnormalities. 1, Enlarged or distorted cochlea; 2 and 5, enlarged ampullae; 3, shortened crus commune; 4, canals thin or misshapen; 6, otoliths absent, or thin or large crystals.

by X irradiation (9–16 d) (ref. 8). Details of the behaviour and effects on the vestibule of some of these offspring are shown in Table 2. The lesions in the balance defective animals are similar to those of balance defective offspring of genetically histidinaemic mothers⁶ (Fig. 1). Some of the offspring without balance defects show subclinical levels of vestibular damage (Table 2*a*), as has been observed in some offspring histidinaemic mothers⁶. Offspring of untreated, metabolically normal females of the same stock show no clinical or subclinical lesions (Table 2*b*). Thus high maternal levels of histidine or its derivatives, however produced, are necessary for the production of balance defective offspring.

It is also possible to modify plasma histidine levels in histidinaemic (*his/his*) mothers during this critical second week of pregnancy. Histidine levels can be increased two to

must lie above a 'saturation level' beyond which histidine levels are no longer relevant in determining the proportion of affected offspring. This conclusion is reinforced by the observations, previously described⁶, that in any one litter there may be affected and unaffected offspring although all will have been exposed to the same maternal metabolites. In these circumstances other factors must be involved in the variation within and between litters. The low histidine diet, however, decreased the proportion of balance-defective offspring from 26% to 3%. In the lower range maternal histidine levels are therefore critical in determining the frequency and severity of balance defective offspring.

High levels of other amino acids (Table 1) during pregnancy do not have the same effect as histidine. Mice, homozygous for the gene *Pro-I^b* (refs 9–11) have <5%

Table 3 Effect of a 2% histidine-supplemented diet, normal diet and low histidine diet, during the second week of pregnancy on the offspring of homozygous (*his/his*) mothers

Diet	No. of litters	No. of animals	Severe	Effect on balance	Normal	% Affected	χ^2	P
(-)2% Histidine	23	79	12	Mild	57	27.8	1.15	ns
Normal	22	94	11	13	70	25.5	—	—
Low histidine	20	65	0	2	63	3.1	25.2	<0.001

Genetic background of the animals, diet and scoring of the balance defect were as for Tables 1 and 2. The number of offspring with their balance severely affected, mildly affected and unaffected were tested by χ^2 test for both the 2% histidine-supplemented diet and the low histidine diet against the expected numbers on the normal diet.

ns, not significant

threefold further by a 2% histidine-supplemented diet and, of more importance, histidine levels can be reduced to about 10% (approaching *his/+* or *+/+* levels) by a low histidine diet (Table 1). The behaviour of offspring of mothers who received these diets is shown in Table 3. The 2% histidine-supplemented diet (which doubles the plasma histidine levels) did not significantly increase the proportion of balance defective offspring.

The histidine in untreated *his/his* mothers therefore,

proline oxidase activity with consequent high levels of proline in their tissues. We have found that such prolineaemic mothers on a normal diet or fed a 2% proline-supplemented diet do not produce balance-defective offspring although their plasma proline is as high as plasma histidine in histidinaemics. Similarly, pregnant C57BL mice were fed on either 5% or 10% lysine or phenylalanine-supplemented diet in the, critical, second week of pregnancy. These diets increase plasma lysine or phenylalanine

up to about $3 \mu\text{mol ml}^{-1}$, comparable with the level of histidine in histidinaemics. The offspring from treated mothers had no balance defects, deafness or vestibular damage (although litter size was reduced considerably on the 10% lysine or phenylalanine supplemented diets). The balance defect in offspring of histidinaemic mothers is therefore not the result of a simple increase in any amino acid to these concentrations during pregnancy although other, untested amino acids could be effective.

Table 4 Abnormalities of the inner ears of $-/-$ animals born to histidinaemic mothers after egg transfer

Animal	Damage to inner ear		Right	
	Left			
	Canals	Otoliths	Canals	Otoliths
1	Abnormal	Altered	—	—
2	Normal	Altered	—	—
3	Normal	Altered	Normal	Altered
4	Normal	Altered	Normal	Altered
5	Normal	Altered	Normal	Altered
6	Normal	Normal	Normal	Altered
7	Normal	Normal	Normal	Normal
8	Normal	Altered	Normal	Normal
9	Normal	Normal	Normal	Normal
10	Normal	Normal	—	—
11	Normal	Normal	Normal	Normal
12	Normal	Altered	Normal	Altered
13	Normal	Normal	Normal	Normal
14	Normal	Normal	Normal	Normal
15	Normal	Normal	Normal	Normal

Blastocysts were transferred 3.5 d *post coitum* into the uteri of recipient females 2.5 d *post coitum* by the technique of McLaren and Michie¹³. Animals 1–12 were C57BL $-/-$ eggs transferred into *his/his* hybrid females (50% Peru/50% C57BL). Animals 13–15 were Peru $-/-$ eggs transferred into *his/his* Peru females. All animals behaved normally and were not deaf. The ears were prepared and scored as in Table 1.

Our previous work⁵ suggested that *his/+* offspring from histidinaemic mothers were less severely affected than *his/his* offspring. Because the only offspring obtainable from histidinaemic mothers are of *his/his* or *his/+* genotypes, we investigated this offspring-genotype effect further by $+/+$ egg transfers¹² into pregnant histidinaemic mothers. The transferred eggs had coat colour markers distinguishing them from the recipient's own eggs. No $+/+$ offspring with any visible balance defects or deafness were found. The ears of two groups of litters, however, showed subclinical damage (Table 4). This is significantly different from both $+/+$ and *his/+* animals born of heterozygous mothers (which show no subclinical damage) but

These genes could operate through the maternal uterine environment or through the susceptibility of the offspring.

We tested this possibility by (1) putting the *his* allele into a different genetic background, and (2) relaxing selection for the balance defect. First, the Peru stock was crossed to C57BL mice, and offspring from histidinaemic mothers of this hybrid stock were examined (Table 5). There was a significant reduction in balance defective offspring (37% to 16%) from the hybrid stock although maternal histidine levels were the same in the hybrid and Peru strains (Table 1). Preliminary results of backcrosses of the *his* allele into C57BL suggest that the proportion of balance-defective offspring can be reduced still further. Similarly, a line in which selection was relaxed for 4 yr (about 12 generations) has reduced the proportion of balance defective offspring from more than 80% to below 10% although, again, the plasma histidine levels are the same in the relaxed and selected stocks. The nature of these modifying effects is being investigated. Preliminary results on reciprocal crosses between the 'high' and 'low' penetrance lines showed a low penetrance for both types of mother. This strongly indicates foetal susceptibility as the major component in penetrance difference and 'dominance' of 'low' over 'high' genes.

It is possible to draw some firm and some tentative conclusions from our observations. The developmental abnormality, whether subclinical or overt, is caused by abnormally high histidine or imidazole derivatives during the second week of pregnancy. These may be due to the genotype of the mother or to her diet. The teratogenic effect can be ameliorated (and possibly eliminated) by dietary management of genetically histidinaemic mothers during the critical period. The alternative explanation that the teratogenic effect is caused by the reduction of flux through histidase in histidinaemic mothers, is unlikely as heterozygotes have no reduction in flux (A. Whitehouse and G.B., in preparation) but can produce affected offspring when fed the 8% histidine supplemented diet.

The effect of the offspring genotype at the *his* locus on the penetrance and severity of the damage seems to be: *his/his* > *his/+* > $+/+$. This is, however, difficult to reconcile with the fact that histidase, which differs in adults in three genotypes, is not measurably different until about a day after birth. Furthermore, treatment of *his/+* mothers during the third week of pregnancy was ineffective. A possible explanation involves the assumption that, contrary to other evidence, there are continuing metabolic effects on ear development after birth when the offspring's

Table 5 Effect of genetic background on the proportion of balance defective offspring produced by histidinaemic parents

Genetic background	Affect on balance								Affect on hearing					
	No. of litters	No. of animals	severe	No. of mild	normal	% Affected	χ^2	<i>P</i>	severe	No. of mild	normal	% Affected	χ^2	<i>P</i>
Peru	12	57	16	5	36	36.8	—	—	18	5	34	40.4	—	—
Hybrid Peru/C57BL	12	51	5	3	43	15.7	11.3	<0.02	8	1	42	17.6	12.4	<0.01

Some *his/his* mice from the Peru stock were crossed to C57BL and the offspring were intercrossed to produce *his/his* animals which were used as hybrid (50% Peru/50% C57BL) parents. The animals were scored for balance as in Table 1 and tested by χ^2 as in Table 3.

suggests that offspring genotype at the *his* locus in some way modifies the severity of the maternal effect.

The original strain of Peru mice, in which the *his* allele occurred, had been selected for high penetrance of the balance-defective character without the knowledge of the presence of the allele and of the effect of maternal histidinaemia^{5,13,14}. It is possible that this strong selection for several years may have accumulated modifying genes increasing the penetrance of the maternal effect on balance.

metabolism is significantly different in the three genotypes.

There is firm evidence that the genetic background is a contributing factor. This effect is, however, not due to any alteration in the histidine levels and it seems likely that it is offspring susceptibility rather than maternal uterine environment which is affected by modifying genes.

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Survival of dinoflagellate blooms in the western English Channel

DURING the summer of 1976, for the second successive year, blooms of the dinoflagellate *Gyrodinium aureolum* Hulbert have occurred at the western entrance to the English Channel. The influence of tidal mixing on the temperature structure of the water column and the availability of light and nutrients for phytoplankton growth in this region have already been described^{1,2}. Here we report a frontal movement in response to the cycle of tidal mixing induced by the alternation of neap and spring tides, and suggest that plant populations both in surface water on the stratified side of the front and in the thermocline are dependent on the associated periodic release of nitrate and inorganic phosphate from the cold, nutrient-rich, bottom layer. We also describe the effects of phytoplankton on nutrient gradients across the thermocline.

To use ship time as efficiently as possible, both surface and subsurface measurements of temperature, salinity and chlorophyll *a* were recorded continuously while steaming^{2,3}. Surface water was sampled through the ship's hull from a depth of 2 m, and sub-surface water was pumped up ($\sim 100 \text{ l min}^{-1}$) from about 20 m through polyvinyl chloride tubing (4 cm internal diameter) attached to a V-fin depressor with a span of 1.3 m. Standard automated analytical techniques⁴ were used to monitor silicate and nitrate in surface or subsurface water. Nitrate was chosen in preference to inorganic phosphate because, after the spring diatom outburst, the low (< 2) nitrate-N to phosphate-P ratios in surface waters suggest that nitrate is the limiting nutrient for the growth of dinoflagellates⁵. An example of records across a frontal boundary, where particularly high levels of chlorophyll *a* were encountered, is given in Fig. 1. Vertical profiles for the same water properties were obtained at a series of stations by lowering the salinity-temperature-depth measuring system and the inlet tube the pump into the water. Data on zooplankton distribution, based on samples taken from the outflow of the pump, will be reported elsewhere.

By comparing the records for temperature and chlorophyll *a* obtained just before (July 27–28, 1976) and just after (July 31–August 2, 1976) spring tides, the changes that occurred during

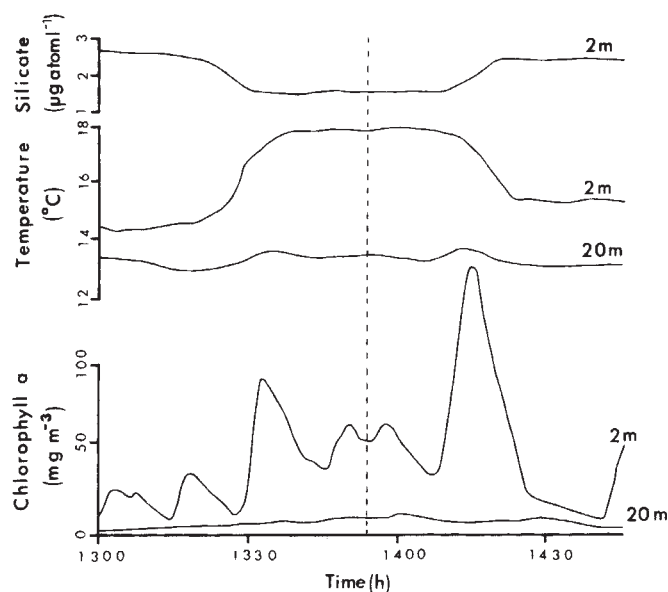


Fig. 1 Surface (2 m) and subsurface (20 m) records for chlorophyll *a*, temperature and silicate (surface only) across a frontal region (49°10'N, 4°48'W, August 3, 1976). At 1352 h the ship altered course to make a second crossing of the front (speed ~ 9 knots).

the period of increased tidal mixing are clearly apparent (Fig. 2). In the former period the temperature front, as defined by the 15–16°C contours, was close to the French coast. Then, as bottom turbulence increased at spring tides, the thermocline was eroded from below and a large area of cold well-mixed water ($< 13^\circ\text{C}$) was produced around the French coast. The frontal region between well-mixed water and stratified water had been displaced further towards the centre of the English Channel. At one place north-west of Ushant the vertical mixing was sufficiently severe to produce a reduction of nearly 6°C in surface temperature.

The limits of frontal movement define an area where phytoplankton populations tend to be dispersed at spring tides but experience conditions suitable for growth as the water column stabilises at neap tides¹. The most notable feature of the chlorophyll distribution maps, however, is the large area on the stratified side of the front where the concentration of chlorophyll *a*, which was already $> 16 \text{ mg m}^{-3}$, appeared to increase during the period of strong tidal streaming. In the most dense patches of *G. aureolum* the water was chocolate brown in colour, and lines of white or yellowish brown detritus (Fig. 3) were again observed¹. Associated with these scum lines, and also indicative of zones of water convergence, were reddish patches of *Noctiluca scintillans* (Macartney) Ehrenb., and large numbers of the copepod *Anomalocephala patersoni* Templeton. Further to the west, where greater water depths and weaker tides make possible a deeper and more stable thermocline, and extending across the edge of the continental shelf, surface chlorophyll *a* values were generally less than 0.5 mg m^{-3} .

Vertical sections of water properties through the front show the position of the phytoplankton bloom in relation to the temperature structure and nutrient distribution (Fig. 4). While taking samples of phytoplankton from the thermocline, differences in the concentration gradients for nitrate and silicate were observed. In a typical vertical profile these are difficult to demonstrate because of the time lag of the analyser system and because most of the change between surface and bottom mixed layers occurs in a few metres within the thermocline. But by taking advantage of the calm conditions and leaving the hose inlet and temperature probe at a fixed depth corresponding to the mean depth of the chlorophyll-rich layer, it was possible to measure levels of the two nutrients at various positions across the temperature gradient as the passage of internal waves (period 5–10 min) caused slow up and down movement of the thermocline⁶. Figure 5 shows that while there is approximately a one to one correspondence between the temperature and silicate traces, the nitrate values increase significantly only

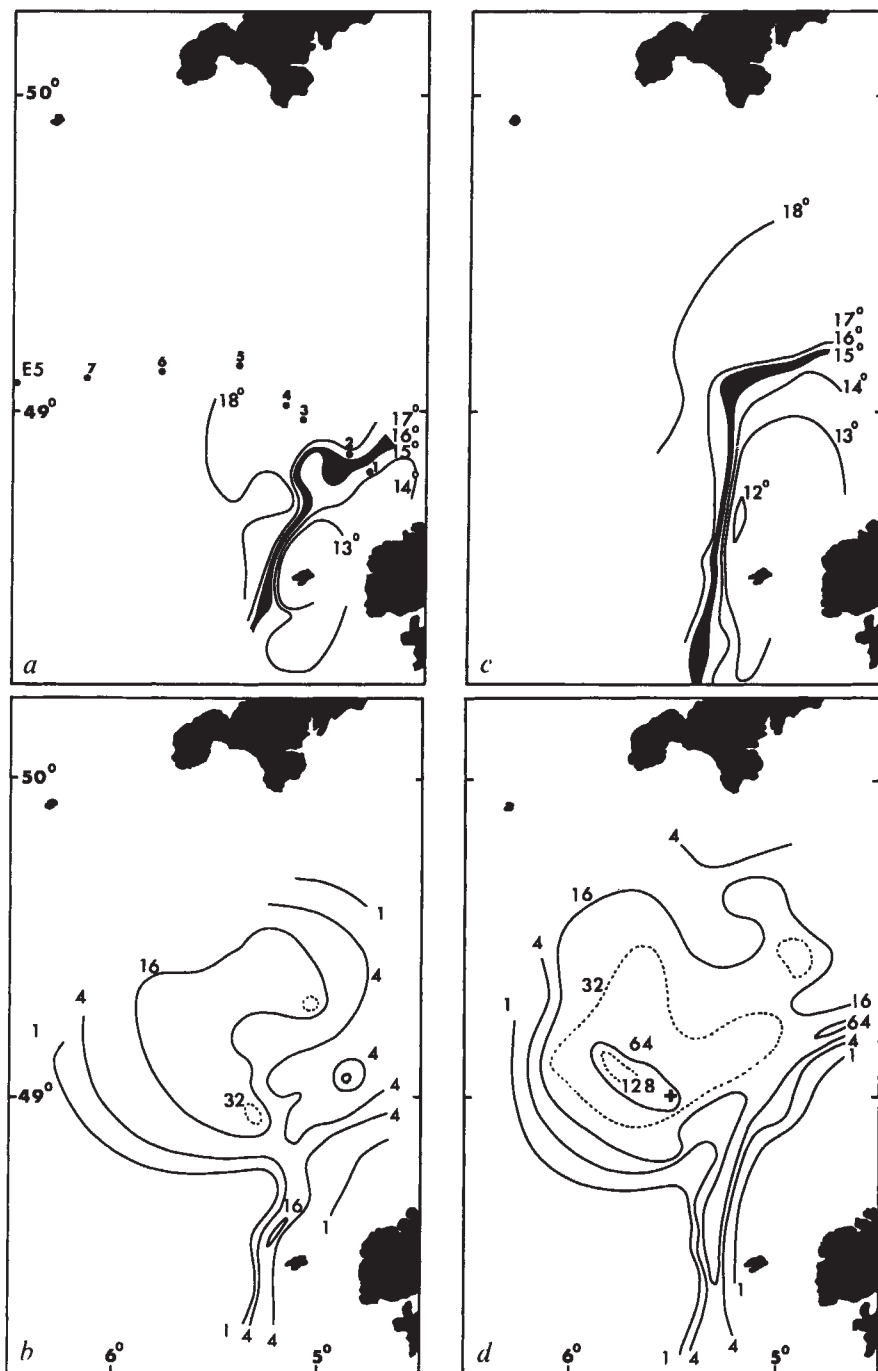


Fig. 2 *a*, Surface temperature ($^{\circ}\text{C}$); *b*, surface chlorophyll *a* (mg m^{-3}) distributions for July 27–28, 1976. *c*, Surface temperature ($^{\circ}\text{C}$); *d*, surface chlorophyll *a* (mg m^{-3}) for July 31–August 2, 1976 (+ marks the position at which Fig. 3 was taken).



Fig. 3 Surface scum at 49°00'N, 5°30'W Fig. 2*d*, 1150 h GMT, August 9, 1976, from 100 m.

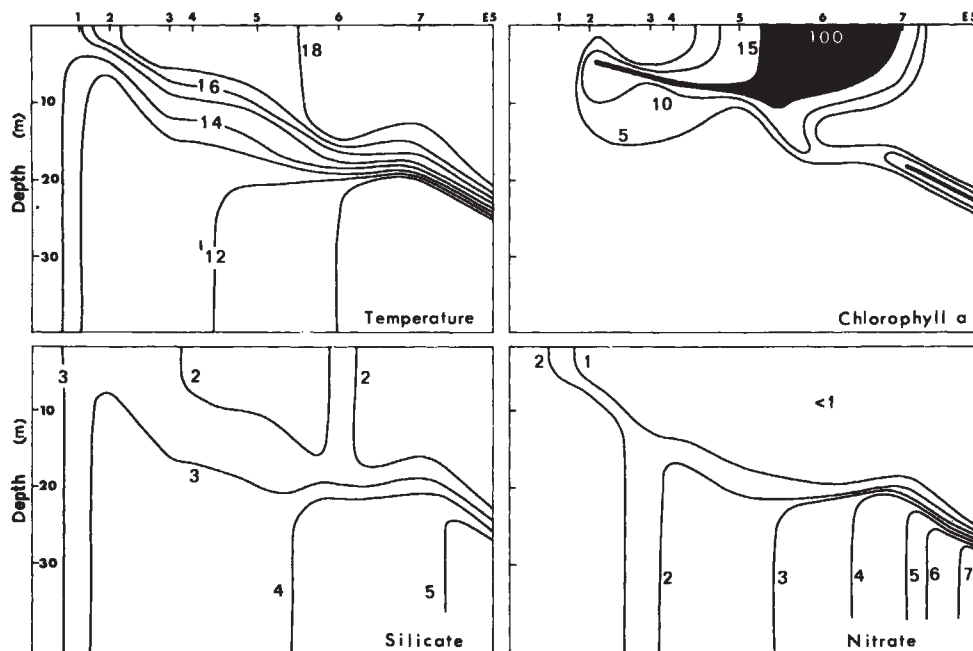
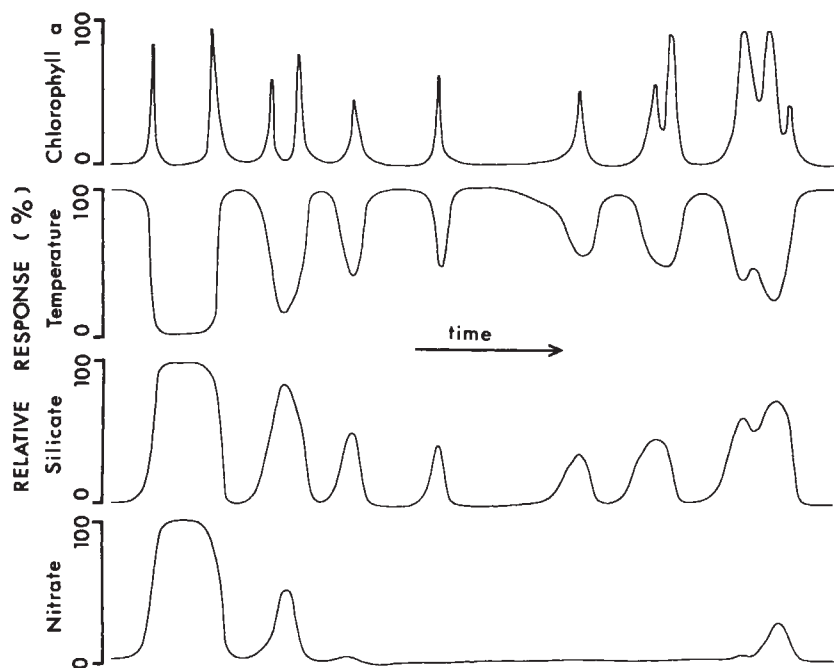


Fig. 4 Vertical sections from temperature ($^{\circ}\text{C}$), chlorophyll *a* ($\mu\text{g atom l}^{-1}$), and silicate and nitrate ($\mu\text{g atom l}^{-1}$) from mixed (station 1) to well stratified water (station E5). The station positions are shown in Fig. 2a. Extreme values for inorganic phosphate, the distribution of which was similar to nitrate, were 0.13 and $0.71 \mu\text{g atom l}^{-1}$ for surface and bottom water at station E5.

Fig. 5 Records from station E5 (1450–1610 h GMT, August 10, 1976) for nitrate, silicate, temperature and chlorophyll *a* at the mean depth of the thermocline (25 m). Each is presented on a scale of percentage change between values for the surface and bottom mixed layers (Fig. 4).



near the base of the thermocline and below the chlorophyll peak. Analysis⁷ of discrete samples indicates that the vertical distribution of inorganic phosphate is similar to that of nitrate.

Dinoflagellates, unlike diatoms, do not have a high requirement for silicate and this is reflected in the relatively high surface values for silicate (Fig. 4)⁸. But the survival of dense populations of these organisms, in both the thermocline and frontal surface water, must depend on an injection of nitrate and inorganic phosphate and this, we suggest, takes place at the base of the thermocline during spring tides at fortnightly intervals. Where the thermocline is relatively shallow, the increased bottom mixing at this time will also affect surface water. Thus the eastern boundary of the more persistent part of the dinoflagellate bloom is marked by the position of the front at spring tides, whereas the western limit is controlled by a deeper and more stable thermocline through which there is little or no exchange of water properties between the surface and bottom mixed layers. Any upward movement of the plant cells during spring tides will minimise the risk of thermocline populations being mixed downwards into the poor light regime of the bottom mixed layer, as well as help to explain the very high surface chlorophyll values which are more than ten times those found in

typical spring outburst conditions². We predict the occurrence of similar phytoplankton blooms and gradients in nutrient concentrations on other parts of the Shelf region of the British Isles where comparable hydrographic conditions exist; for example in the North Sea near the Farne Islands, to the east of Orkney and Shetland, and to the west of the Outer Hebrides⁹.

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Local reactivation of Triton-extracted flagella by iontophoretic application of ATP

IN spite of recent progress in ultrastructural and biochemical studies of cilia and flagella¹, the mechanism of their movement is still poorly understood. Much circumstantial evidence, however, supports the sliding microtubule model of ciliary and flagellar motility² which involves active sliding between axonemal microtubules to provide the motive force for bending. For example, ATP-dependent sliding of microtubules has been demonstrated in the Triton-extracted axoneme lightly digested by trypsin³. To produce a bend in the sliding microtubule model, there should be local differences in the amount of sliding along the axoneme. Morphological evidence indicates the presence of such differences in the bent region of cilia⁴. We now report experiments involving local, brief reactivation of Triton-extracted sea urchin spermatozoa by iontophoretic application of ATP. Our results support the idea of sliding within the axoneme as the basis of flagellar bending.

Spermatozoa from the sea urchin, *Anthocidaris crassispina*, were extracted as described before⁵. The extracted spermatozoa ('models') were placed in a 'reactivating solution without ATP' (for composition, see legend to Fig. 1) and a single model was held by attaching its head to a polylysine-coated glass needle⁶ mounted on a micromanipulator. A glass capillary microelectrode, filled with a solution containing 10 mM ATP and with a resistance of about 500 M Ω , was used to activate the model locally by iontophoresis. The movement of the model was observed by an inverted microscope (Nikon MD) fitted with phase-contrast optics (objective: Nikon BM 40 $\times$), and was filmed on Kodak Double-X or Plus-X 16 mm film at 32–64 frames per s with a Bolex H16 ciné camera.

When the tip of the ATP electrode was placed close to the axoneme and ATP was applied as a negative pulse lasting 100–300 ms, the axoneme typically showed a localised, brief (less than 3 s) oscillation in the vicinity of the electrode. The frequency of observed oscillation was 2–7 Hz.

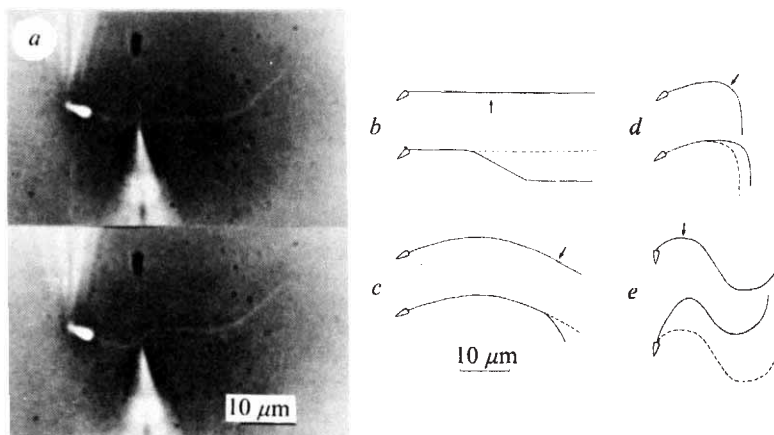
The amount of ATP, expressed in nC, necessary to bring about the oscillation, varied with the specimen and also with the electrode used, but a few nC was usually sufficient. With this amount of ATP, it was possible to induce the response in any region of the axoneme, but the response was always localised and did not propagate beyond 5–9 μ m of the electrode. This shows that any region along the length of the flagellum is capable of active bending⁷, and also that a truly local application of ATP was achieved with our method. With less ATP, the duration of induced oscillation was shorter, and below a certain level a full cycle of movement was no longer obtained. Instead, the axoneme changed its form by a localised unidirectional movement of the region near the electrode (Fig. 1). By a few repetitive applications of the small amount of ATP to the same place, however, it was possible to induce one or more complete cycles. In other words, it was possible to break down a beat cycle of a segment of flagellum into a few steps.

The small amount of ATP applied to a more or less straight region of the axoneme typically elicited a local bending response (Fig. 1a–c). Usually, a pair of similar bends was formed in opposite directions (Fig. 1a and b) except in the distal region where only a single bend was formed (Fig. 1c). There was no fixed relation between the positioning of the electrode relative to the axoneme and the direction of bending, which seemed to depend on the orientation and other conditions of the axoneme. The above result is just what the sliding microtubule model predicts, if it is assumed that ATP causes a unidirectional sliding near the electrode and there is resistance to sliding outside the range of action of ATP. Diagrams illustrating this interpretation are shown in Fig. 2.

When the small amount of ATP was applied to the bent region, the bend moved some distance distally as the region directly under the electrode straightened (Fig. 1d). If the electrode was moved distally and ATP was applied to the newly formed bend, the bend was displaced further distally; this procedure could be repeated several times until the bend disappeared from the distal end of the flagellum. If there were two bends in the shape of an S, an application of ATP to the proximal bend had no visible effect on the distal bend: only the proximal bend moved distally (Fig. 1e). Similarly, ATP applied to the distal bend caused a distal displacement of this bend only, with no influence on the proximal bend.

The amount of ATP necessary to unbend the axoneme with consequent displacement of the bend tended to be lower than that necessary to bend the straight region. In

Fig. 1 Response of Triton-extracted flagella to local application of ATP. Spermatozoa of *Anthocidaris crassispina* were first suspended in 25–50 volumes of Ca-free artificial seawater buffered to pH 8.0 with Tris-HCl. Two drops of this suspension were added to 5.0 ml of extracting solution consisting of 0.15 M KCl, 4 mM MgSO₄, 0.5 mM EDTA, 0.5 mM mercaptoethanol, 0.04% (w/v) Triton X-100, 2 mM Tris-HCl, pH 8.0, at room temperature (25–28 °C), and were swirled gently for 30 s–1 min. The resultant suspension of extracted spermatozoa ('models') was transferred to 2 volumes of 'reactivating solution without ATP', which consisted of 0.15 M KCl, 2 mM MgSO₄, 0.5 mM EDTA, 5 mM dithiothreitol, 2% (w/v) polyethylene glycol and 20 mM Tris-HCl, pH 8.0, and was used immediately for local reactivation experiments. A single model was attached by its head to a polylysine-coated glass microneedle obtained by dipping the tip in 0.1% (w/v) polylysine, followed by a brief rinse in distilled water. At the end of local reactivation experiments, each model was exposed to bath application of 10–100 μ M ATP, and models that failed to propagate normal waves were discarded. a, Prints from a 16-mm ciné film, showing a model both before and about 0.4 s after an iontophoretic application of ATP (0.13 nC). Note the position of the ATP-electrode and the induced local bending of the axoneme. b–e, Tracings from ciné films, each showing a model before (upper) and after (lower) application of ATP. Each arrow indicates the position of the ATP-electrode. The form of the axoneme before application of ATP is also shown superimposed on lower traces (dashed lines). The amount of ATP and the interval between the time of ATP pulse and the time of recording of the response were: b, 1.4 nC, 300 ms; c, 1.2 nC, 210 ms; d, 0.6 nC, 330 ms; and e, 0.8 nC, 350 ms, respectively.



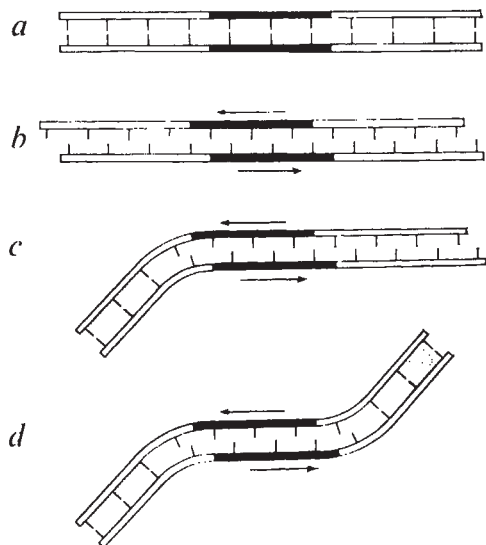


Fig. 2 Diagrams showing how a local active sliding (indicated by arrows) between two flexible filaments is converted into a bending. *a*, Before sliding. *b*, If there is no resistance to sliding between the filaments, active sliding does not lead to bending. This is presumably what happened to the trypsin-treated model of Summers and Gibbons³. *c*, If there is a region with resistance to sliding (stippled) on one side of the active region, a bend will be formed between these regions, compare Fig. 1*c*. *d*, If there is resistance to sliding on both sides of the active region, two bends will be formed in opposite directions, compare Fig. 1*b*.

one experiment, for example, bending of the proximal region required ATP at 2.0 nC while 0.2 nC was sufficient to straighten the same region. This indicates the possibility that once a bending wave is formed, it can be propagated in a concentration of ATP which is lower than is necessary to initiate a new wave. To test this, we placed the model in a solution containing 8 μ M ATP. This concentration has been known to be lower than that required for the reactivation of bending, although enough to relax the "rigor waves"⁸. An iontophoretic application of ATP to the model in this solution induced a bending wave which was propagated down the flagellum. Propagated waves were also produced when the glass needle holding the sperm head was vibrated by a light tap at its handle. This also depended on the presence of a low concentration of ATP, since no such propagation was observed without ATP in the bathing solution. Our results indicate that a localised sliding between axonemal elements leads to bending, and also provide observations that may be important in understanding the mechanism of flagellar wave formation and propagation. The iontophoretic technique has proved useful for studying flagellar movement; it will be used to advantage in the studies of various aspects of cell motility at the cellular or organellar level.

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New approach to screening drugs for activity against African trypanosomes

THE African trypanosomes are unicellular eukaryotic parasites responsible for serious diseases in man (such as sleeping sickness) and domestic animals (such as nagana in cattle). The chemotherapy of these diseases is unsatisfactory¹. Only a few drugs are available; these have serious side effects, drug resistance is a problem and "new drugs for clinical use are too expensive to develop and produce"² using classical empirical screening methods. We report here how knowledge of trypanosome metabolism has led to a new method for screening drugs against trypanosomes. This could provide a short-cut to new drugs or drug combinations.

The bloodstream forms of African trypanosomes are completely dependent on glycolysis for their energy supply, because the biogenesis of a mitochondrial respiratory chain is suppressed in the vertebrate host^{3,4}. As Fig. 1 shows, aerobic glycolysis proceeds through the Embden-Meyerhof pathway similar to the glycolytic pathway of the host with the exception that pyruvate is excreted into the host's bloodstream as it cannot be metabolised further because long slender bloodstream trypanosomes lack both a functional Krebs cycle^{4,5} and lactate dehydrogenase⁶. The NAD⁺ reduced in the glyceraldehyde phosphate dehydrogenase step is reoxidised by a mitochondrial⁷, CN⁻-insensitive L-glycerol-3-phosphate oxidase system* (boxed in Fig. 1), which is not present in the vertebrate host.

When the oxidase is completely inhibited by anaerobiosis or salicylhydroxamic acid (SHAM)⁸, the bloodstream trypanosomes convert glucose into equimolar amounts of pyruvate and glycerol. According to Fig. 1, the net synthesis of ATP in this pathway should be zero. It has been demonstrated, however, that this is not the case and that high SHAM concentrations neither block trypanosome motility *in vitro*⁹ nor affect the course of a fulminant *Trypanosoma brucei* infection in rats¹⁰. This has led to the conclusion that the scheme in Fig. 1 is oversimplified and that the bloodstream trypanosomes contain an, as yet unknown, extra

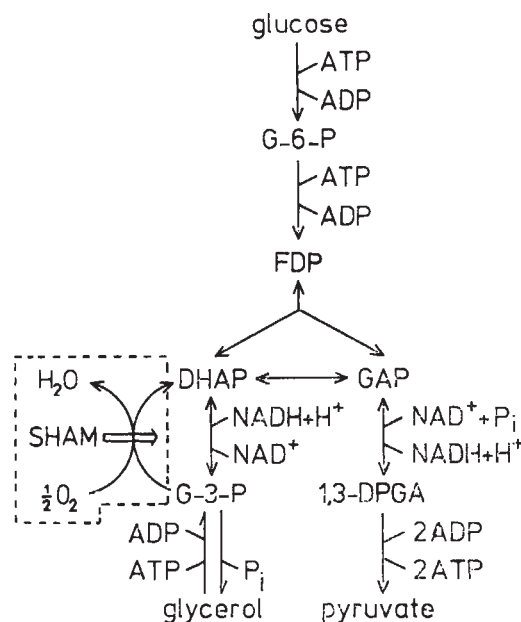


Fig. 1 Glycolysis in bloodstream trypanosomes. G-6-P, glucose-6-phosphate; FDP, fructose-1,6-diphosphate; GAP, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone phosphate; G-3-P, L-glycerol-3-phosphate; 1,3-DPGA, 1,3-diphosphoglycerate.

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Table 1 Effect of SHAM on the motility of bloodstream form *T. brucei* incubated in a glucose-glycerol mixture

Substrate present (mM)		SHAM added (1 mM)	Motility after		
Glucose	Glycerol		5 min	15 min	30 min
25	0	—	++++	+++	+++
25	0	+	+++	+++	++
25	0.5	+	+++	+++	++
25	1.0	+	+++	++	+
25	2.0	+	++	+	+
25	5.0	+	+	—	—
25	10.0	+	+	—	—

Heparinised rat blood infected with *T. brucei* EATRO 427 was diluted 1:1 with 50 mM phosphate buffer, pH 8.0, containing 45 mM NaCl and 50 mM glucose. Samples (1 ml) were incubated at room temperature with various amounts of glycerol plus SHAM. Samples were taken at the times indicated and examined by phase contrast microscopy within 30 s for motility.

pathway for the reoxidation of NADH, which allows the synthesis of 1 mol of ATP per mol of glucose when the glycerolphosphate oxidase is blocked^{9,10}. An inhibitor of this pathway, combined with SHAM, might therefore be lethal to the trypanosome, though either inhibitor alone would be ineffective¹⁰. We have found that SHAM and glycerol represent such a lethal combination. We were led to these experiments by previous work by Ryley¹¹, who has shown that glycerol inhibits glucose utilisation by trypanosomes in anaerobic conditions.

Table 1 shows that SHAM alone does not abolish motility of trypanosomes incubated in blood with glucose as substrate, but that motility progressively decreases when increasing amounts of glycerol are included in the mixture. In other experiments with purified trypanosomes glycerol concentrations as low as 1 mM were sufficient to immobilise

the trypanosomes. A more quantitative analysis of the effects of glycerol is given in Fig. 2. In this experiment the rate of glycolysis was monitored by measuring pyruvate production. Glycerol has little effect on glycolysis in the absence of SHAM, but in the presence of SHAM 5 mM glycerol is sufficient to inhibit pyruvate formation by 99%. Half-maximal inhibition was obtained with 0.8 mM glycerol (0.4 and 1.2 in two other experiments). This inhibition by glycerol can be explained by product inhibition of the putative alternative pathway for NADH reoxidation^{9,10}, but other explanations remain to be excluded.

As it is possible to obtain plasma SHAM levels that completely block the glycerolphosphate oxidase¹⁰ and glycerol levels exceeding 5 mM (ref. 12), our results predict that it should be possible to kill *T. brucei* also in the living rat with SHAM+glycerol. This prediction has been verified¹³. Glycerol administration might also potentiate the therapeutic action of suramin, a drug used in the treatment of human sleeping sickness, as the glycerolphosphate oxidase system is the only trypanosomal enzyme reported to be inhibited by low suramin concentrations¹⁴. Finally, our results show how insight into trypanosome metabolism could lead to the development of drug combinations that would not have been detected in a conventional screen for trypanocidal drugs, because neither of the drugs alone has measurable trypanocidal action. It might be useful to extend present drug screening procedures to include screening of drugs in the presence of SHAM or glycerol.

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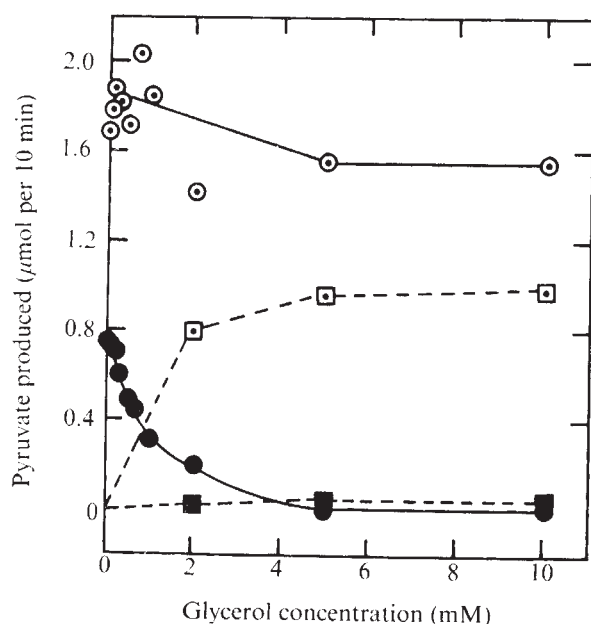


Fig. 2 Effect of SHAM on pyruvate production by *T. brucei* incubated in glucose-glycerol mixtures. Purified trypanosomes (0.7 mg of protein) were incubated at 37 °C in a 1-ml reaction mixture containing 50 mM sodium phosphate buffer, 45 mM NaCl, pH 8.0, with glucose and/or glycerol as substrate. SHAM was added as a solution in dimethylformamide; an equal volume of dimethylformamide was added to the control incubations. Samples were deproteinised with perchloric acid at zero time (to correct for any pyruvate in the trypanosomal suspension) and after 10 min of incubation. After neutralisation the supernatants were assayed for pyruvate. Further details of the methods used are given in ref. 9. Circles, trypanosomes incubated with 10 mM glucose plus 0-10 mM glycerol; squares, trypanosomes incubated in glycerol alone. Open symbols, no SHAM added; closed symbols, plus 1 mM SHAM.

Feeding of *Daphnia* on *Chlamydomonas* and *Chlorobium*

AMONG investigations of filtration mechanism and food selectivity of filter feeders¹⁻⁷, Gophen *et al.*⁶ have used double labelling to show the preferential absorption of bacteria by *Ceriodaphnia reticulata* (Jurine) in long term experiments. Lampert⁷, using the same technique but with a 15-min exposure, reported a coefficient of selection greater than 1 for bacteria ingested by small daphnids in the presence of mixed food. I present here the results of a study of

Table 1 Content of food source, suspension and incubation times

No.	Bacteria		Algae		Incubation times
	Cells per ml ($\times 10^7$)	Cells per c.p.m.	Cells per ml ($\times 10^5$)	Cells per c.p.m.	
1	5.0	172	13.7	22	0.5; 2; 5; 10;
2	7.5	490	5.7	1	0.3; 1; 2; 5;
3	2.5	1,306	15.9	31	0.3; 1; 1.5; 2.5; 5;
4	6.0	530	10.4	10	5; 10; 20; 40; 60;
5	5.0	903	7.2	10	5; 10; 20; 40; 60;

the feeding mechanism of *Daphnia magna* (Strauss) during short exposure to bacteria, algae or a mixture of the two.

Chlamydomonas reinhardtii (Dangeard), cultured in Bristol's medium and labelled with ^{14}C (ref. 6) and *Chlorobium phaeobacteroides* Pfennig cultured on Pfennig's medium and labelled with ^3H (ref. 6) served as food sources. They were collected and resuspended in filtered lake water and 0.3 ml of each suspension was solubilised in 5 ml of Aquasol Universal LSC cocktail, and radioactivity was determined in a Packard liquid scintillation counter. The cell concentration of the suspension was determined by counting the algae on a haemocytometer and by measuring the specific absorbance of bacterial chlorophyll at 715 nm. The correlation between absorbance at 715 nm and cell number was obtained by incubating serial dilutions of the cell culture in Agar-shake tubes containing Pfennig's medium and counting the colonies that developed after 3 weeks. By this method an absorbance of 1 was equivalent to 4.35×10^7 cells per ml (B. Z. Cavari, unpublished results).

The volume of cells was measured and their wet weight was calculated by assuming a density of 1.0. Values of 1.5×10^{-4} μg per cell and 1.6×10^{-8} μg per cell were found for *Chlamydomonas* and *Chlorobium*, respectively.

Several hundred newborn daphnids were pipetted from the culture into filtered lake water. They were starved for 24 h in the dark at room temperature (22–24 °C). Each experiment was carried out as follows: ten animals were introduced into tubes containing algae, bacteria or mixtures of both and incubated for

different times (Tables 1 and 2). All experiments were done at room temperature in the light. With exposure for more than 5 min, the test tubes were mixed gently every 5–10 min. At the end of the exposure, the daphnids were removed as quickly as possible by sieving through a nylon net and washed with filtered lake water. They were then transferred into scintillation vials and 5 ml of Aquasol was added. After 24 h, radioactivity was determined. The samples were then sonicated in an ultrasonicator (MSE) at high power, in ice for 2 min. This treatment disintegrated the organisms. Counts after sonication were higher by a factor of 1.5 (± 0.2) for ^{14}C and a factor of 1.1 (± 0.1) for ^3H . Therefore all samples were sonicated before counting.

The ingested biomass is shown in Table 2. The average bacterial biomass ingested in each experiment was 1.3 (± 0.3) times greater when the animals were fed on mixed food compared with bacteria alone. In contrast, the ratio between ingested biomass of algae by the daphnids that were fed mixed food compared with those fed algae alone was 0.5 (± 0.1).

It is likely that in the presence of *Chlorobium* alone the rejection rate is higher than in the presence of the relatively large algal cells. Conversely the presence of bacterial cells in the mixed food source lowered the ingested biomass of *Chlamydomonas*. As Table 2 shows when fed bacteria alone, the daphnids ingested $0.1\text{--}4.2 \times 10^{-3}$ μg per animal, and $0.1\text{--}5.7 \times 10^{-3}$ μg per animal when exposed to mixed food. The similar ranges of ingestion of algae were $0.1\text{--}12.3$ μg per animal when the daphnids were fed on algae, and $0.1\text{--}10.7$ μg per animal when given mixed food.

Table 2 Ingested fresh-weight in μg per ind and selectivity coefficients (see text)

No.	Incubation time (min)	Bacteria		Algae		Bacteria and Algae		S_1 $^3\text{H}^*$	S_2 $^3\text{H}^*$
		10^{-3} μg per ind	μg per ind	10^{-3} μg per ind	μg per ind	10^{-3} μg per ind	μg per ind		
1	0.5	0.3	0.6	0.3	1.6	0.3	1.6	1.6	1.6
	2	0.2	1.9	0.1	0.2	0.1	0.3	0.3	1.5
	5	0.4	5.9	0.2	1.2	0.2	0.2	0.2	0.6
	10	0.6	4.3	0.2	2.3	0.2	0.4	0.4	0.3
	Average	0.4	3.2	0.2	1.3	0.2	0.1	0.1	0.3
2	0.3	0.5	0.3	0.4	0.1	0.4	0.1	0.1	0.3
	1	0.2	0.2	0.4	0.1	0.1	0.1	0.1	0.3
	2	0.3	0.1	0.9	0.1	0.1	0.2	0.2	0.3
	5	0.3	0.2	0.5	0.1	0.1	0.1	0.1	0.4
	Average	0.3	0.2	0.6	0.1	0.1	0.1	0.1	0.3
3	0.3	2.2	5.6	2.9	4.1	2.9	4.1	0.1	0.3
	1	2.5	5.6	5.1	3.1	5.1	3.1	0.2	0.6
	1.5	4.2	8.2	2.6	3.9	2.6	3.9	0.2	0.3
	2.5	2.0	8.5	5.1	10.7	5.1	10.7	0.1	0.2
	5	3.5	12.3	5.7	5.9	5.7	5.9	0.1	0.4
4	Average	2.9	8.0	4.3	5.5	4.3	5.5	0.1	0.3
	5	0.9	1.8	3.4	0.5	3.4	0.5	0.1	0.9
	10	2.2	0.7	1.6	0.8	1.6	0.8	0.5	0.3
	20	1.2	1.9	2.3	1.2	2.3	1.2	0.1	0.3
	40	1.0	1.1	2.6	0.8	2.6	0.8	0.1	0.4
5	60	2.2	1.6	2.0	0.7	2.0	0.7	0.2	0.4
	Average	1.5	1.4	2.4	0.8	2.4	0.8	0.1	0.3
	5	0.4	0.4	0.3	0.1	0.3	0.1	0.1	0.6
	10	0.4	0.2	0.3	0.1	0.3	0.1	0.2	0.6
	20	0.4	0.3	0.4	0.2	0.4	0.2	0.1	0.3
Average	40	0.4	0.3	0.5	0.1	0.5	0.1	0.2	0.5
	60	0.1	0.4	0.1	0.1	0.1	0.1	0.04	0.3
	Average	0.3	0.3	0.3	0.1	0.3	0.1	0.2	0.5
	Total average							0.1	0.1
	$\pm$ standard deviations								

ind, Individual.

* Selective coefficients.

Selectivity coefficients were calculated from the data. The average value of S_1 ^3H (for animals fed separate food sources) was $0.2 (\pm 0.1)$ while the average S_2 ^3H value (for animals fed mixed food) was $0.5 (\pm 0.1)$.

Lampert⁷, using an exposure time of 15 min and mixed food only, showed that young daphnids are remarkably selective in their feeding habits. Table 2 shows that feeding rate is highest during the shortest exposure times and lower during longer exposure times. In all cases, however, no selectivity was found.

There are two possible reasons for this difference: (1) *Daphnia* may digest bacteria faster than algae, or (2) the accumulation, in long-term experiments, of bacterial material as opposed to algal material may be due to the shorter retention time of ^{14}C -labelled substances (algal source), which might be used for intensive metabolic activities such as respiration. The results presented here and derived from long-term experiments with *Ceriodaphnia*⁶ and *Daphnia*⁷ suggest the following processes. Initially ^{14}C -labelled algal materials are ingested in larger amounts during normal feeding. Later, the rapid digestion of bacterial materials results in the accumulation of ^3H -labelled substances. I conclude that the rate of ingestion is influenced by the size of the particles present in natural or artificial bodies of water. This is important in ecosystems containing suspended particles of a diversity of sizes.

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Chromophore mobility in bacteriorhodopsin

THE sole protein found in the purple membrane of *Halo-bacterium halobium* contains retinal covalently bound by means of a protonated Schiff base linkage to lysine^{1,2}. The only established function of this protein is to act as a light-driven proton pump producing a transmembrane proton gradient, which is coupled to ATP synthesis in the living organism^{3,4}. In so far as the retinal prosthetic group resembles that found in the visual pigment of vertebrates, rhodopsin, the name bacteriorhodopsin has been widely adopted for this protein³. Considerable work has been done on the biochemistry and photochemistry of the purple membrane, both for its intrinsic interest as a unique photosynthetic membrane and with respect to the identification of possible mechanistic relationships between its photochemical cycle and that of the visual process. We report here a study of the later stages of the photochemical cycle by flash photometry with plane polarised light. Our results indicate conformational change in the bacteriorhodopsin molecule at the site of the visible chromophore.

There has been speculation as to whether rotational motion of the visual pigment is an essential link in the signal chain in the visual process⁴. It has been demonstrated that rhodopsin molecules undergo rapid Brownian rotation within the membrane^{4,5} and the theory has been proposed (but criticised on energetic grounds⁶) that rotation about an axis parallel to the plane of the rod disks ('tumbling') could enable rhodopsin to act as a transmembrane ion carrier⁴. In contrast, bacteriorhodopsin exists in a crystalline form in the purple membrane⁷. Consequently, Brownian diffusion of individual molecules

would be expected to be severely restricted, and this has been confirmed^{8,9}. But during a study¹⁰ of the temperature dependence of the photochemical cycle in aqueous suspensions we were surprised that the measured rotational relaxation times gave an activation energy for the medium viscosity (see below) which differed significantly from that characteristic of water. If the protein chromophore is rigidly bound within a crystal lattice which has dimensions comparable with those of the membrane, then rotational relaxation would be exclusively a consequence of the tumbling of the individual fragments, constrained only by the viscosity of the water in which they were suspended. This consideration induced us to carry out a more extensive study of rotational mobility.

The bacteriorhodopsin photochemical cycle contains a sequence of phototransients of which one, *M*(410), has been shown to have the retinal Schiff base unprotonated¹¹. The decay of *M*(410) is linked kinetically to the reappearance of the ground state *BR*(570), with a lifetime at room temperature in the millisecond region¹². Rotational mobility was investigated by measurement of the time dependence of the decay of linear dichroism of the absorption band of *M*(410) at 410 nm. By means of a conventional flash photometer⁹, purple membrane samples were excited with μs light flashes filtered successively through a 500-nm cutoff filter and a linear polariser with its optical axis vertical (laboratory *a* axis, Fig. 1). Light transmission was then viewed in the *c* direction with a beam

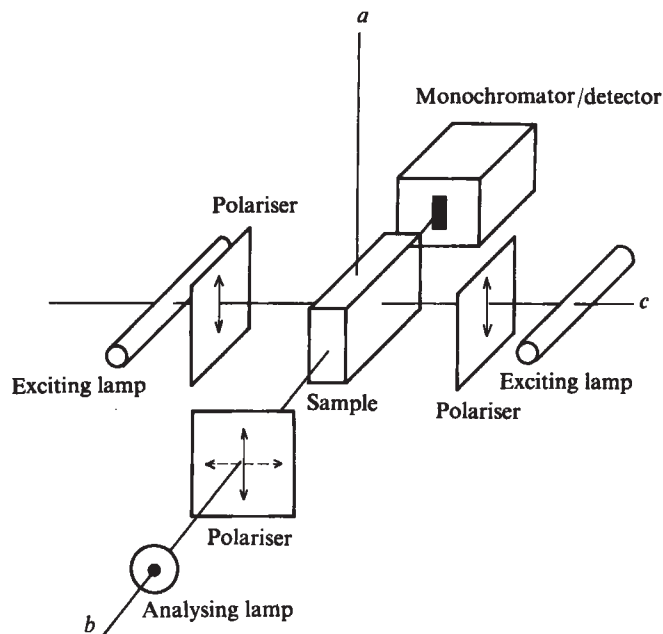


Fig. 1 Optical layout of flash photometer for measurement of relaxation of linear dichroism in phototransient absorption.

polarised alternately parallel and perpendicular to the exciting light. The time dependence for absorption of the two polarised analysing beams is given by¹³

$$A(t)_{\parallel} = 5/3 + \alpha\beta\exp(\theta t) \quad (1)$$

$$A(t)_{\perp} = 5/3 - \frac{1}{2}\alpha\beta\exp(\theta t) \quad (2)$$

where θ is the rotational rate constant, proportional to temperature and inversely proportional to medium viscosity and size of the rotating species ($kT/V(r)\eta$), β is a function of the optical transition moments of the chromophore absorbing the exciting light and the species being viewed (*M*(410)), and α is a randomisation factor ($\alpha < 1$) which encompasses instrumental artifacts in addition to unidentified (non-Brownian) causes of depolarisation¹³. Total time-dependent absorption is then given by equations (1) and (2) multiplied by a weighting

factor which reflects the decay of the population of the absorbing species being viewed. By defining a function $R(t) = (A(t) - A(t)_\infty) / (A(t)_0 + 2A(t)_\infty)$ which is analogous to anisotropy commonly used for fluorescence polarisation¹⁴, a purely exponential expression for dichroic decay is obtained.

$$R(t) = 3/10\alpha\beta \exp(\theta t) \quad (3)$$

This is independent of the decay kinetics of the phototransient.

As reported previously⁹, little decay in dichroism can be detected at room temperature in aqueous suspensions of purple membrane during the lifetime of $M(410)$. Transient lifetimes, however, increase markedly with decreasing temperature ($Q_{10} \approx 3$) and adequate first-order plots were obtained (s.d. $< 10\%$) consistent with equation (3). The results are presented in Fig. 2(A) in the form of an Arrhenius plot of the viscosity dependent ratio T/θ . The best straight line yields an energy of activation for microviscosity, E , of 7.4 ± 0.3 (s.d.) kcalorie mol^{-1} . This is significantly higher than that for water¹⁵, and shows the presence of rotational freedom in addition to that of fragment tumbling. Purple membrane, when treated with the protein crosslinking reagent glutaraldehyde, continues to undergo reversible photochemical cycling. The lifetimes are slightly increased, facilitating a more extended Arrhenius plot. This (see Fig. 2(B)) yields $E = 4.4 \pm 0.04$ (s.d.) kcalorie mol^{-1} , consistent with published values for water¹⁵. The size calculated¹⁶ for the membrane fragments (diameter = $0.5 \mu\text{m}$) assuming them to simulate oblate ellipsoids is in excellent agreement with published values (diameter = $0.5 \mu\text{m}$)¹, affording a test for the validity of the theory.

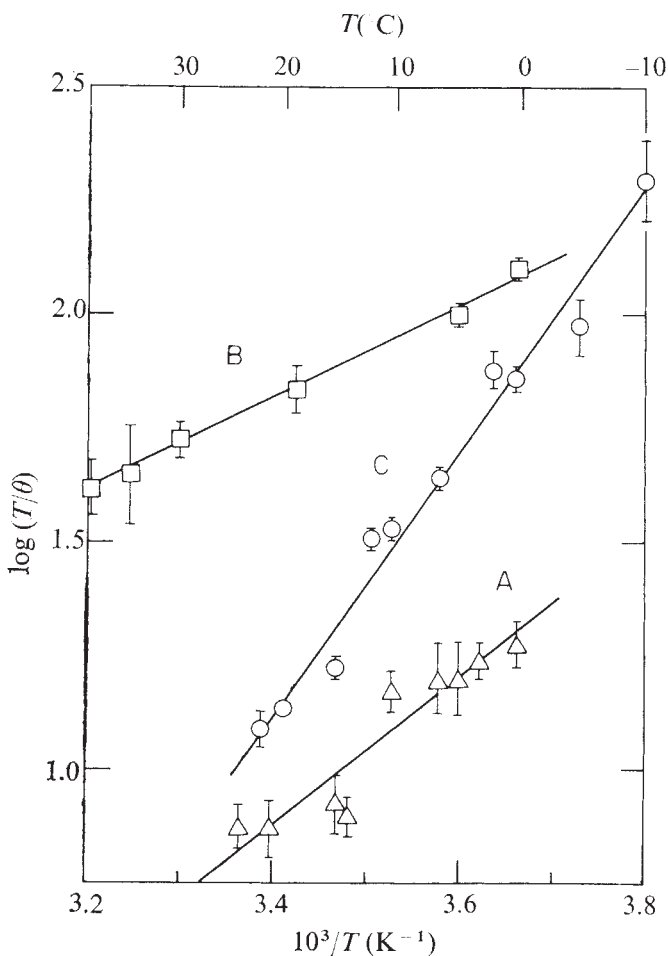


Fig. 2 Arrhenius plots of the viscosity-proportional function T/θ . Bars represent standard deviation in the measurement of θ . Δ , Membrane fragments in water; $\square$, membrane fragments pretreated with glutaraldehyde and suspended in water; $\circ$, membrane fragments in a 20% polyacrylamide gel.

Both untreated and glutaraldehyde-fixed purple membranes were incorporated into a 20% polyacrylamide gel by *in situ* polymerisation of acrylamide monomer added to aqueous purple membrane suspensions. At this concentration the membrane fragments may be expected to be completely immobilised. The photochemistry seems unchanged, with photo-transient yields and lifetimes in the polymer indistinguishable from those in pure water. For the glutaraldehyde-fixed sample no dichroic relaxation was detectable within the whole temperature range (detection limits at 20 and 0°C , respectively, $\theta = 10^{-1}$ and 10^{-2} s^{-1}). But for the unfixed polymer first-order dichroic decay was observed, yielding $E = 13 \pm 0.05$ (s.d.) kcalorie mol^{-1} (Fig. 2(C)).

The results presented here show that the chromophore of bacteriorhodopsin is not totally immobilised within the purple membrane. The significant rotational rate constants found, 20 s^{-1} at room temperature, are too high to be attributable to rotation of individual protein molecules *in toto* within the crystal lattice, and must indicate internal conformational change. It is tempting to speculate, based on the close coincidence of E for rotational mobility and that found for the formation of $M(410)$ (12 kcalorie mol^{-1})¹², that the deprotonation step which produces this intermediate is controlled by intramolecular movement involving the retinylidene group (which simulates Brownian motion). The establishment of internal mobility within bacteriorhodopsin suggests that it might be profitable to look for an analogous process, in contrast to the criticised tumbling mechanism, in the transmission of the visual signal in rhodopsin subsequent to the primary *cis-trans* isomerisation.

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Three-chain merokeratin from wool may be a fragment of the microfibril component macromolecule

WOOL fibres consist mainly of long cortical cells filled with microfibrils, 7-8 nm diameter, embedded in a matrix. The microfibrils are composed of proteins with a lower sulphur content and a higher α -helix content than wool; the matrix proteins have a higher sulphur content than wool and no α helix^{1,2}. When reduced and carboxy-methylated low-sulphur proteins from wool (SCMKA) are separated by starch gel electrophoresis, there are two

main bands, component 7 (molecular weight 46,000) and component 8 (molecular weight 51,000)^{1,11}, and it has been suggested that the microfibrils are composed of regularly arranged macromolecules (molecular weight 143,000) consisting of two component 7 molecules and one component 8 molecule⁵.

Merokeratin A₁ is a rod-shaped, low-sulphur protein fragment (dimensions ~2×21 nm, molecular weight 50,000 to 60,000, 64% α helix) which dissolves when reduced wool is treated with trypsin⁶. In the experiments described here, A₁ has been prepared in a variety of conditions and dissociated into its component chains using urea, sodium dodecyl sulphate (SDS) and, in some experiments, mercaptoethanol. The chain molecular weights have been measured by polyacrylamide gel electrophoresis (PAGE) and compared with the molecular weight of undissociated A₁. The results imply that A₁ consists of three protein chains, which suggests that it may be part, approximately one third of the length, of the microfibrillar macromolecular component protein.

Similar merokeratins have been prepared from low-sulphur wool proteins⁷⁻⁹ and from epidermal keratin proteins¹⁰⁻¹³ by protease digestion.

Different amounts of trypsin and different temperatures and times of digestion were used in four methods of treating reduced wool (Fig. 1*a-d*). The wool debris was removed by centrifugation and the supernatant protein solutions were exposed to the air to reoxidise the thiol groups to disulphide bonds; the solutions were first diluted to favour the formation of intramolecular, interchain crosslinks¹⁴. Each solution was acidified to precipitate the protein. When the precipitates were redissolved in pH 8.0 buffer and fractionated on a Sepharose 6B column, the elution volume of the major retarded peak, fraction A₁, was independent of the severity of the trypsin treatment. The yield of A₁ was lower when the mildest conditions were used for the digestion (Fig. 1*a*). A small amount of protein (V) is eluted at the void volume of the column. Fraction B* is a heterogeneous mixture of low-sulphur peptides crosslinked by disulphide bonds; its composition varies with the method of preparation (S.S.L., unpublished results).

The most concentrated A₁ fraction from each of the Sepharose fractionations in Fig. 1 was denatured with urea and SDS in the presence and in the absence of mercaptoethanol. PAGE showed either two or three intensely stained, partially resolved bands, and one or more less intense bands (Fig. 2). Standard proteins were denatured in the same way and run in parallel to calibrate the gels. The molecular weights of the main bands were between 14,000 and 17,000. The proteins from the more severe trypsin digestions were slightly shorter than those from the less severe digestions. The calculated values of the molecular weights in Fig. 2 are probably within $\pm 10\%$ of the true values¹⁵.

All the gels showed a band of protein which migrated more slowly than the main bands, which had a molecular weight of about 31,000, or about 29,000 when digestion was at 40 °C. The band was most prominent when A₁ was prepared by the mildest digestion procedure and denatured with no reducing agent present (Fig. 2*e*). Its susceptibility to reducing agent (see Fig. 2*a*) suggests that the protein of the band may consist of two proteins with molecular weights between 14,000 and 17,000 crosslinked by one or more disulphide bonds. The lower intensity of the 31,000 or 29,000 bands when digestion was carried out under more severe conditions (Fig. 2*f-h*) suggests that the crosslinks are in the ends of the molecules prepared by mild digestion, and that these ends are removed in the more severe digestions. A weakly stained 31,000 or 29,000 band persists even when A₁ is denatured with a reducing agent present (Fig. 2*a-d*). This could be explained if some cystine

residues were converted to non-reducible lanthionine or lysinoalanine residues during the alkaline reduction of wool before the trypsin treatment^{16,17}. The small peak of protein with a molecular weight of 49,900 in Fig. 2*e* suggests that after the mildest digestion there may be a small amount of material in which three protein chains with molecular weights between 14,000 and 17,000 are crosslinked. Two of these proteins crosslinked together and to

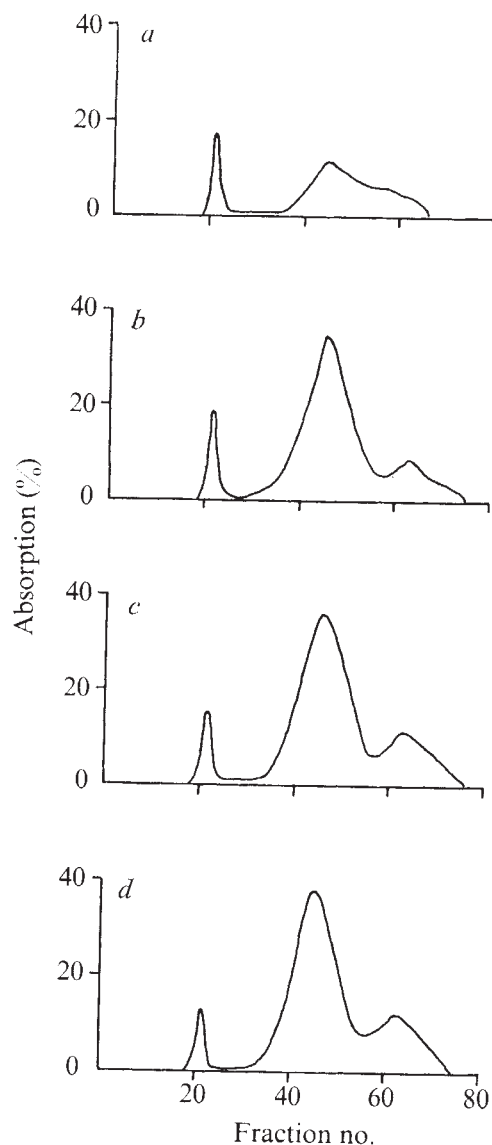


Fig. 1 Sepharose 6B chromatography of protein fragments dissolved from reduced wool by trypsin under various conditions. 2.0 g samples of purified Lincoln wool were reduced for 18 h at room temperature in 1.0 l of 0.1 M Na₂HPO₄, 0.2 M thioglycolic acid adjusted to pH 10, rinsed four times with 100 ml 0.1 M Na₂HPO₄ at pH 8.0, transferred to 40 ml of the pH 8.0 buffer, and treated with trypsin. Temperatures, times of digestion and wool-enzyme ratios (w/e) were as follows: *a*, 0 °C, 30 min, w/e 250:1; *b*, 0 °C, 60 min, w/e 50:1; *c*, 20 °C, 60 min, w/e 50:1; *d*, 40 °C, 60 min, w/e 50:1. After the digestions the trypsin was inhibited by 8 ml 0.25% phenylmethylsulphonylfluoride¹⁸ in 50% v/v isopropanol-water. The digests were centrifuged at 50,000 *g* for 30 min. The supernatants were diluted to 1.5 l with 0.1 M Na₂HPO₄, pH 6.0, and exposed to the air in shallow dishes for 22 h at room temperature¹⁴. In each experiment, the reoxidised protein was precipitated by adjusting the pH to 4.8. The precipitates were collected by centrifugation at 1,000 *g* for 10 min, and redissolved in 5 ml 0.1 M Na₂HPO₄ pH 8.0 buffer. The solutions were dialysed for 2 h against the same buffer, clarified at 150,000 *g* for 10 min, applied in turn to the column, and eluted with the pH 8.0 buffer. Fraction A₁ is the major retarded peak. V and B* elute at fractions 21 and 62 respectively. The percentage absorption of the eluent from the column was measured at 254 nm in an LKB Uvicord I.

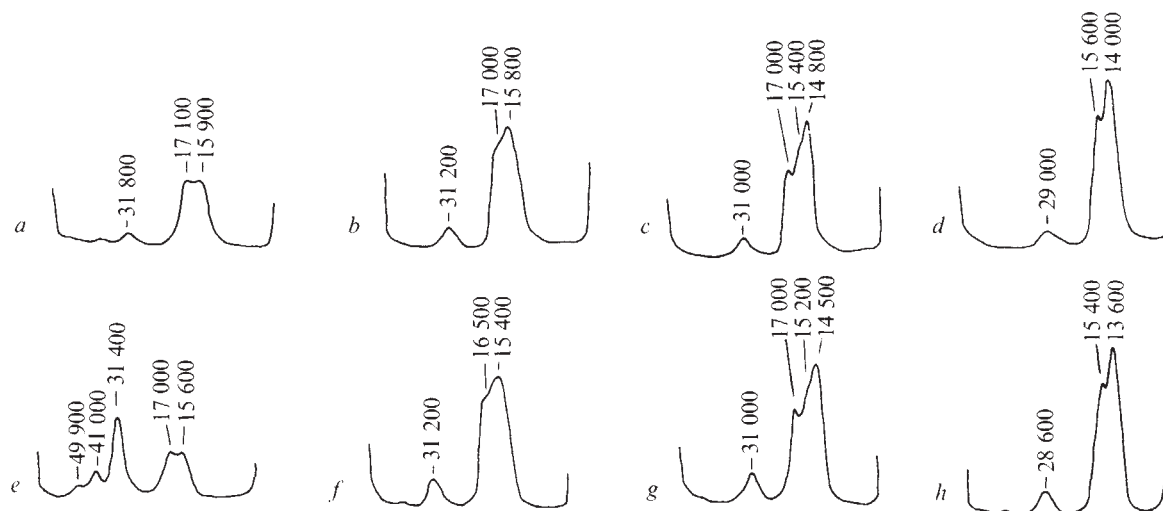


Fig. 2 Polyacrylamide gel electrophoresis (PAGE), in the presence (a-d) and in the absence (e-h) of reducing agent, of Fraction A₁ prepared by trypsin digestion of reduced wool in various conditions. a, 0 °C, 30 min, w/e (wool-enzyme ratio) 250:1; b, 0 °C, 60 min, w/e 50:1; c, 20 °C, 60 min, w/e 50:1; d, 40 °C, 60 min, w/e 50:1; e, 0 °C, 30 min, w/e 50:1; f, 0 °C, 60 min, w/e 50:1; g, 20 °C, 60 min, w/e 50:1; h, 40 °C, 60 min, w/e 50:1. a-d, The most concentrated A₁ fraction from each chromatograph in Fig. 1a-d was diluted to 0.2 mg ml⁻¹ with 0.1 M Na₂HPO₄ buffer at pH 8.0, mixed with an equal volume of 8 M urea, 2% sodium dodecyl sulphate (SDS), 2% mercaptoethanol (ME) in distilled water and heated to 45 °C for 1 h. After cooling, one drop of the solution was applied to a 10% polyacrylamide gel cross-linked with 0.6% bis-acrylamide. After electrophoresis in 0.1% SDS, 0.1 M Na₂HPO₄, pH 8.0, the gels were stained overnight with 0.25% Coomassie brilliant blue dissolved in 20% trichloroacetic acid, destained in a mixture of 3 volumes acetic acid, 2 volumes methanol, 35 volumes water, and scanned with a Joyce-Loebl densitometer. Bovine serum albumin, pepsin, trypsin, myoglobin, haemoglobin, lysozyme and chymotrypsin were denatured in the same way and run at the same time as the A₁ fractions. The molecular weights of the bands from the A₁ fractions were estimated by interpolation on the line obtained when log (molecular weight) of the standards was plotted against the mobility during electrophoresis. e-h, The most concentrated fractions from Fig. 1a-d were denatured as described above except that ME was omitted. The molecular weights of the component chains of A₁ were estimated as before, except that the standard proteins were also denatured without reducing agent.

a large peptide would account for the 41,000 molecular weight band in Fig. 1e.

The molecular weight of fraction A₁, prepared by trypsin digestion of reduced wool at 20 °C for 60 min with a 50:1 wool-trypsin ratio (A₁²⁰), is 50,000 to 60,000 measured at several pH values in the ultracentrifuge, using the Ehrenberg method; or 53,600 calculated from the sedimentation coefficient (3.22 s), the diffusion coefficient (4.83×10^{-7} cm² s⁻¹) and the partial specific volume (0.69 ml g⁻¹) at pH 6.0 using the Svedberg equation⁶. It is not possible to say definitely whether A₁²⁰ has three or four component chains when these molecular weights of undissociated A₁²⁰ are compared with the molecular weights of the component chains from denatured A₁²⁰. Fig. 2c and g show that the proteins from A₁²⁰ responsible for the two major peaks and the shoulder between them, are not present in equal amounts. Also, all the molecules in one PAGE band do not necessarily have the same composition. Therefore, each undenatured molecule may be composed of three chains, each slightly different in weight. The chain weights (and compositions) are not necessarily the same in different undenatured A₁²⁰ molecules. This heterogeneity is an expected result of the limited proteolysis method of preparation.

Assuming that the staining of each band is approximately proportional to the amount of protein in the band, Fig. 2c and g show that the proteins from A₁²⁰ responsible for the two major peaks and the shoulder between them, are not present in equal amounts. Also, all the molecules in one PAGE band do not necessarily have the same composition. Therefore, each undenatured molecule may be composed of three chains, each slightly different in weight. The chain weights (and compositions) are not necessarily the same in different undenatured A₁²⁰ molecules. This heterogeneity is an expected result of the limited proteolysis method of preparation.

The peptide bonds involving the carboxylic acid groups of the 26 lysine and 31 arginine residues in each undenatured A₁²⁰ molecule⁶ are not hydrolysed by trypsin during the preparation. This implies that the component chains of A₁²⁰ are held in a rigid conformation throughout most of their length by hydrogen bonds and other secondary valence forces, so that the complexes which are necessary for hydrolysis to occur cannot form between trypsin molecules and the chains. The low yield of fraction

A₁ (7.5% of the weight of the wool)⁴ implies that, after reduction, the chains in the remainder of the microfibril component macromolecule may be more flexible and therefore digested to small peptides by trypsin. The role of the rigid part of the macromolecule may be to interact with the rigid parts of similar, neighbouring macromolecules to ensure their correct alignment and staggering in the microfibril, and experiments are in progress to investigate the aggregation properties of A₁. The flexible part of the macromolecule may be partly responsible for the mechanical resilience of the fibre, and it may interact with the high-sulphur proteins of the fibre matrix⁸.

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matters arising

Brain tryptophan in rats on a high fat diet

INASMUCH as the article by Hutson *et al.*¹ constitutes a serious criticism of the data reported from our laboratories over the past three years²⁻⁶, we would like to make the following reply:

● In 1974, some of us⁴ examined the validity of the various methods then available for measuring free tryptophan in blood. We described fully that the equilibrium dialysis method, performed at 37 or at 0 °C, gave identical results for serum-free tryptophan and for serum NEFA levels. The values for NEFA were the same as those measured before dialysis; this test was done with sera containing high (1 meq l⁻¹) and low (0.2 meq l⁻¹) amounts of NEFA. We thus ruled out the potential problem of lipolysis during dialysis over two years ago, as Hutson *et al.* admit only parenthetically (page 143).

● In this same paper⁴, we tested the method of Knott, Curzon, and their colleagues, which involves separation of free tryptophan using Amicon filter cones centrifuged at 800g. In this method, no attempt is made to control pCO₂, and thus serum or plasma pH, during dialysis. Tryptophan binding is extremely sensitive to pH changes; using this method, the pH can run as high as 8.0 or more, as observed by Madras *et al.*². We note that in the article by Hutson *et al.*¹, on page 143, pH values of 7.75, 7.78, and higher were recorded, which affirms the non-control of pH in this method, and confirms the findings of Madras *et al.*².

● Hutson *et al.*¹ claim that they obtain similar findings when they assay either plasma or serum. But they fail to provide data on free tryptophan values in serum; instead they give data for serum UFA—and assume that free tryptophan behaves similarly. This assumption must be proved in each new experimental situation.

● Finally, we would like to note the following: (1) Hutson *et al.*¹ provide little information about the diets consumed by their animals. What were the protein and fat contents of these diets? How can the authors call the standard laboratory chow diet a "control diet?" (2) How much food was consumed by each group? (3) Why was plasma total tryptophan lower in the group eating

the high fat diet? It seems to us that this "diet" experiment is uninterpretable as it is presented.

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HUTSON, KNOTT AND CURZON REPLY—We fail to see that the essential point of our letter is seriously affected by any of the issues raised by Fernstrom, Munro and Wurtman. They refer to the work of Madras *et al.*² in which rats on a high-fat diet apparently had high serum free tryptophan not associated with high brain tryptophan. Our own results lead us to question the physiological relevance of these findings as large amounts of unesterified fatty acids (UFA) can be formed by plasma or serum of fat-fed rats *in vitro* and this may lead to the liberation of tryptophan from albumin after blood collection.

Taking the points raised in their letter in order:

● In quoting the finding of Madras *et al.*² that UFA did not increase on dialysing serum at 37 °C our use of the grammatical convenience of parentheses was not intended in any way as a criticism of the validity of this result. However our own findings¹ suggest that UFA may have already increased during the preparation of serum from fat-fed rats.

● As already explained¹ the small pH differences between plasmas of control and fat-fed rats would have had negligible effects on free tryptophan concentration.

● It would be most surprising if UFA changes did not cause free tryptophan changes in rat serum as in plasma. We find a significant positive correlation ($P < 0.01$, $n = 16$) between percentage

free tryptophan and UFA of human sera.

● We did not provide detailed information on the food presented to the rats or on the amount they ate, because this had little relevance to our main conclusion—that changes occur *in vitro* in rat plasma and serum taken from fat-fed rats.

In the circumstances, and considering the many findings consistent with free tryptophan influencing brain tryptophan (refs 3-8 are to papers published in 1976) the results of Madras *et al.*² can hardly be interpreted with confidence.

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Dating earthquakes by mud profiles of lake sediments

BEN MENAHEM¹ asserts that the white laminae in the recent sediments of the Dead Sea represent past occurrences of earthquakes, and identifies in a single sediment core all major shocks of the past 2,000 yr. This exhumation of a long abandoned concept²⁻⁶ relies on two fallacious assumptions. First, new springs are assumed to open up in the Dead Sea before an earthquake, and discharge a 'white material' which whitens the lake waters and deposits on the bottom a lamina of a thickness proportional to the earthquake magnitude. Second, the described 170-cm long core from the proximal part of the Jordan delta is assumed to represent the full record of deposition in the past 2,000 yr, and thicknesses of the dark-coloured sequences sandwiched between successive white laminae are assumed to be a measure of earthquake recurrence times^{2,3,6}.

Both these assumptions are incompatible with facts:

(1) No pre-earthquake emergence of new springs nor large fluctuations of flow from existing springs have been

observed in the Dead Sea region^{2,3}. A continuous layer 1–2 mm thick represents a deposition of up to a million tons of dry material^{3,5} (nearly pure aragonite), the derivation of which from springs would require many billions of cubic metres of water, even if very high concentrations are assumed. Such a gargantuan influx and a corresponding rise in lake levels (1 m per influx of 10^9 m³) could not have passed unnoticed³. Likewise, no mud or gas volcanoes supplying CO₂ which would induce precipitation of carbonates were ever reported. For this reason, clearly stated in the previous works^{2,3}, the hypothesis of springs whitening the Dead Sea waters was abandoned in favour of earthquake-triggered landslides^{3,4} or an aseismic chemical process⁵.

Recent study of the Dead Sea⁷ established that 'whitening' occurs when late summer high temperatures trigger a massive precipitation of aragonite from the hypersaline lake waters oversaturated, through evaporation, with HCO₃ and SO₄. The high temperature origin of the aragonite laminae is confirmed by their isotope composition⁸. Also the whitenings of sea waters in other parts of the world has been attributed to biogenic or chemical processes unrelated to seismicity^{9,10}.

(2) 15–20 White laminae were deposited⁷ between 1890 and 1960. Neither these laminae, nor the conspicuous whitening of August 1959, investigated in great detail⁷, can be matched with earthquake records.

(3) Composition, colour and thickness of sediments are controlled by fluctuating patterns of sediments supply, transport and deposition¹¹. The pronounced lateral and vertical variations in layering and thicknesses of the dark, detrital sediments of the Dead Sea are determined by discharge, distance from river inlets, water depths and local patterns of circulation and currents¹². In such conditions, thickness is not a unique function of time and should not be used as a yardstick in estimating time intervals.

(4) The sediment core analysed by Ben Menahem was taken in the proximal part of the Jordan delta (rough co-ordinate 199/129), where rates of deposition range up to 20 mm yr⁻¹ (ref. 12), in contrast to the distal parts where values of ~ 1 mm yr⁻¹ were reported^{7,12}. It is very unlikely therefore, that the 170-cm long core represents the full record of 2,000 yr of deposition. Assuming the present rate of formation of white laminae, 15–20 laminae in 70 yr, it would seem that the core with its 50 white layers represents up to 300 and not 2,000 yr. Incidentally, dating and counts of laminae in the Lisan Marl,

a lacustrine formation deposited in the precursor of the Dead Sea¹³ between 65,000 and 15,000 yr BP indicate annual formation of white layers, implying according to Ben Menahem's supposition, a yearly occurrence of strong earthquakes . . .

(5) Last, but not least, of the objections to Ben Menahem's correlations, is the fact that the uppermost part of the discussed core is missing, having been lost in the process of coring⁷. Thus it is not clear how and why the two uppermost white layers in the core were identified as the imprints of the 1903–06 and 1834 earthquakes.

These arguments amplify and vindicate the reservations clearly stated in studies in which the possible correlation between the white laminae and earthquakes was originally discussed^{2,3,5,6}. These doubts, which led to an early rejection of the hypothesis, are not mentioned in Ben Menahem's paper, which seems to rely solely on a correlation between a sequence of historic earthquakes and an undated sequence of layers in a single and incomplete, random sediment core.

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¹ Ben Menahem, A., *Nature*, **262**, 200–202 (1976).

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³ Shalem, N., *Teva*, **2**, 86–90 (1945).

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BEN MENAHEM REPLIES—Karcz, Neev and Zak have unearthed some counter-arguments^{1,2}, but their quoted figures for core dating, sedimentation rates, lacustrine formation dates, summer high temperatures and Lisan Marl laminae counts are of such obscure origin that all my efforts to trace their sources were fruitless.

The main issues centre, however, on "new facts" which the above authors have marshalled against my "assertions" and "assumptions". The first "fact" is the speculation¹ that "whitening" of the Dead Sea is caused by an alleged high temperature triggering of aragonite precipitation—a process that has never been duplicated elsewhere. Now, according to the statistics of Karcz *et al.*, a "whitening" should occur every 5 yr, which

renders about fifteen such events in the twentieth century alone. Unfortunately, only a single true grand-scale "whitening" was documented during this century³, close to the occurrence of a magnitude 5 earthquake. The reported¹ "whitening" of August 1959 was much weaker and probably caused by a microtremor that passed unnoticed by the low-magnification JER station.

A second 'fact' which is mentioned twice by Karcz *et al.*, is based on the desultory observation¹: "... 15 to 20 laminae were found on shore exposures and on trees which are known to have been drowned in the Dead Sea ~70 yr ago." Besides the question of relevance of this novel 'tree-meter' to our mud-core deposits, this observation¹ implies a repetition of 'whitening' on the average every 3 yr since 1890. Ashbel⁴ on the other hand, counted 2,000 annual grey layers in the mud core of Elazari Volcani⁶.

Another "fact" presented by Karcz *et al.*, which contradicts the published reports of Amiran⁷ and Ashbel⁵, is their dubious statement: "No pre-earthquake emergence of new springs was observed in the Dead Sea region". It has, however, been recently established⁸ that the Dead Sea is sitting on a huge active fault, some 60-km long, with an estimated area of 1,500 km². Its motion, whether abrupt or slow, can and should affect the regular flow of the many springs inside and on the shores of the eastern side of the lake. It may, in addition, cause massive landslides and other topographical changes at the flanks and bottom of the Dead Sea, as already suggested by Shalem⁹.

The exact mechanism by which the fault slip or pre-earthquake creep is coupled to the outbreak of turbidity is immaterial to the correlation under discussion.

There is nothing therefore in the objections of Karcz *et al.* that has bearing on the remarkable correlation¹⁰ of past seismic events to the mud-core white layers. Karcz *et al.* have not added new data or information to the already known reports^{3–7,9} while their use of the "drowned-tree timescale"¹ and the "whitening" speculation¹ leads them to predict an almost everlasting snow-white colour for the Dead Sea.

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reviews

Machiavelli with a computer

Eric Ashby

The Humane Technologist. (Science and Engineering Policy Series.) By D. Davies, T. Banfield and R. Sheahan. Pp. ix+180. (Oxford University: Oxford, 1976.) £4.75.

FOR some inscrutable reason the dust cover of this book carries a puff from the co-founders of the Club of Rome. Do not be put off by this. Any apprehension that the book was sponsored by the body which condoned a diet of exponentials for computers is soon dispelled. The authors courteously dissociate themselves from any such nonsense. They are as well equipped as anyone to compose mathematical models of future scenarios; so it is refreshing and reassuring to find them quoting, as a good example of organisational models, the table of contents of Machiavelli's *The Prince*: "and" they add, "the chapters themselves are commendable succinct runs of these models. There are, however, no rules for the construction of such models except the general one of keeping them simple and avoiding the hopeless pursuit of meaningless quantification."

The theme of Davies and his collaborators is that the technologists' honeymoon is over; society will no longer permit man to do all he is capable of doing. But technologists must not lose heart, for they hold the clues to the solution of our present perplexities. They must not lose heart but they must acquire the skills of "human perception and understanding". In brief, an education which equips the technologist to handle ideas and things should equip him, too, to handle people; the immediate threat to industrial societies is not shortage of energy or depletion of resources, it is the risk of disorder due to stresses in human relations, between management and shop floor, between government and citizen.

Drawing on a wealth of examples and the rich background of a great industry, the authors of this intelligent and sophisticated book examine the uses of models by technologists, with special reference to the circumstances of a democratic society. Enriched with historical allusions from Greek tragedy to medieval geopolitics, the book offers facts and opinions on such models as

the Boston Experience Curve (which relates the cost of a product to the accumulated experience of making it); the constraints which limit the use of such models in industry and government; and the part played in technological innovation by access to investment capital. It ends by an appeal not to regard people as "inanimate thermodynamic assemblies of customers, clients, operators, and educators". Technologists have been slow to face some of the human problems they have created; yet they have (so the authors claim in their last chapter) the right sort of experience to deal with models of these problems: numeracy, familiarity with the effects of error and uncertainty, patience in reconciling concepts with the realities of everyday systems.

The book is entertaining, but the authors quite rightly want something more from the reader than "polite applause" or "stunned silence". It is a book to provoke discussion and it deserves to, for the issues it raises are critical for modern society. Perhaps the most ominous of these issues is the dilemma of what the authors call "top-down" models. Having persuaded the reader that models are important—indeed essential—to the proper deployment of technology, they then distinguish two kinds of models. On the one hand there are "bottom-up" models (they cite as an example a model for traffic flow in Westminster) which can be assembled with other subsolutions into pragmatic hierarchies to provide data for political and social decision-making; going from the particular to the general. Such models may be too empirical to provide guidance for grand strategy. On the other hand there are "top-down" models (such as the Club of Rome and the Hudson Institute indulge in) which are synoptic, and which adopt doctrinal or dogmatic guidelines. Because "bottom-up" models are liable to be difficult to integrate into a hierarchical whole, there is a temptation to adopt "top-down" models, such as the Soviet Union has used for years. Then comes the authors' warning: "a state based on top-down modelling throughout cannot afford to be democratic and must be authoritarian if it is to be effective. We reject absolute authority in politics,

and so we must severely restrict the applicability of top-down modelling." But will society reject absolute authority, if the alternative on the horizon is chaos? To rescue society from having to make this choice is the challenge of this book.

For some years observers of industrial society have stressed the need for innovation in political institutions to match innovation in technology and industry. The only recent political innovation (I use the phrase deliberately) which has emerged in response to this is the multinational corporation. Do we discern in (for example) the oil companies the prototype of international power, if not yet of international government? Such corporations are deeply distrusted by the man-in-the-street; they too, like "top-down" models, are not compatible with democratic government.

The book cleverly leads the reader toward the conclusion the authors intend, namely that there ought to be a "constructive alliance" between technologists and sociologists. But how? Alas, the authors turn to education as the recipe. Education is by its very nature conservative; it is a process analogous to the transfer of genes in heredity: a sort of cultural DNA; not the place to look for such major innovation as is needed. If innovation is to come, its source is more likely to be the political philosophers. The need is for a redefinition of values and a machinery for reconciling conflicts of values. Karl Marx offered one pattern of innovation, but for those who value personal freedom the price to be paid for his pattern is too high. Is some lonely successor to Karl Marx now working in the British Museum on a political philosophy adapted to a post-industrial society? If so, he will find in this book the questions to which democratic societies seek an answer.

One postscript: the Oxford University Press used to be impeccable in its standards of proof reading. The misprints in this book are a disgrace to the Press. □

Eric Ashby (Lord Ashby of Brandon) recently retired from the Mastership of Clare College, Cambridge. He was chairman, from 1970 to 1973, of the UK Royal Commission on Environmental Pollution.

Autonomic nervous system

Structure of the Autonomic Nervous System. By G. Gabella. Pp. viii+214. (Chapman and Hall: London; Halsted: New York, June 1976.) £9.80.

DR GABELLA has produced an informative account of the structure of the autonomic nervous system. It is given in small compass (160 pages), and the author has also restricted his historical introduction to just over a page. The book then examines the structural anatomy of sympathetic ganglia and peripheral sympathetic nerves, including their development and regeneration. Parasympathetic structures, including the ciliary ganglia and the vagus nerve, are then described. The last chapter is devoted to the innervation of organs, including the eye, salivary glands, skin, adipose tissue, gut, the heart, blood vessels, respiratory tract and the genital tracts. The monograph is well supported by 800 references which are helpfully arranged at the end of the book in alphabetical order. The references quoted are recent, and many have been published in the 1970s up to 1975. The information is based on comparative anatomy and there is frequent mention of the species on which the observations have been made.

Thus the book provides well documented information on the structures described, and will be valuable as a source of reference to anyone who is dealing with the structural anatomy of the autonomic nervous system in animals as well as man. The book has, however, some limitations. On the production side it seems unfortunate that the error in binding—the plates have been incorrectly placed—should not have been corrected, so that the publishers have only noted the error with an erratum slip. It is nevertheless a well produced and attractive volume. There are also some deficiencies in its composition: structure has been interpreted as histology rather than gross anatomy, and the book provides only limited information about the anatomical distribution of nerves. Thus description of the variations in the sympathetic outflow and its links with the sympathetic chain ('prefixing' and 'postfixing') are not included. The autonomic innervation of the eye is restricted to a discussion of two tissues only—the iris and the nictitating membrane—and there is no discussion of the eyelid. On the structural side the information is chiefly relevant to the peripheral autonomic nervous system, and details of the central nervous system are limited. So limited are they,

that 'spinal cord' and 'hypothalamus' do not occur in the index and the chapter on the central nervous system (chapter 9) is only 3.5 pages long.

The description of the book explains that it is intended as a basic reference work on the autonomic nervous system for students of, and research workers in, the fields of physiology, pharmacology and neurobiology. As a description of the anatomical structures of the autonomic nervous system at a histological level, it is excellent. But it provides only limited information on the physiological mechanisms involved, and the pharmacology of the system is only briefly discussed, there being no biochemical information throughout the book. Clinical research workers will

also find that it does not provide any information on pathology or the clinical relevance of any of the structures described. It may, however, be argued that this was not the intention of the book.

This book can be strongly recommended as a work of reference for any worker engaged in the study of the autonomic nervous system. It should also be available in libraries, but I feel that its information is too restricted for students or most clinical research workers.

R. H. Johnson

Dr Johnson is Senior Lecturer in the Department of Neurology, University of Glasgow, and Consultant Neurologist at the Institute of Neurological Sciences, Glasgow, UK.

Noctilucent clouds

Noctilucent Clouds. By V. A. Bronsh-ten and N. I. Grishin. Pp. v+237. (Israel Program for Scientific Translations: Jerusalem; Wiley: Chichester, February 1976.) £8.25; \$16.50.

EVER since the first observations in 1885, noctilucent clouds have been a challenge to atmospheric physicists, in particular to those dealing with problems related to the upper atmosphere. Apart from data on meteors, information on the 80-km region, where the clouds are situated, was almost non-existent until rocket measurements became available some twenty years ago. Thus, for about seventy years, noctilucent clouds provided a playground for scientists with skill and imagination.

Noctilucent clouds also differ from most other problems in science in the following respect: during ninety years of research an enormous amount of data on various aspects of the problem has been collected and yet the two main problems remain unsolved: how do noctilucent clouds form and what do they consist of?

Since the early 1950s, V. A. Bronsh-ten and N. I. Grishin have contributed much to our knowledge of noctilucent clouds, and their names are well known to colleagues all over the world. In view of their contact with the problem over such a long timespan, they are in the best possible position to undertake such a comprehensive presentation of the history and state of the art of noctilucent clouds.

The book is obviously meant to cover the subject of noctilucent clouds exhaustively, and in large measure the authors have succeeded in their ambitious goal. The huge amount of information on the history of theories, abandoned or still under consideration, and on observational data and tech-

niques, classifies the book as far the most useful and comprehensive source for students at all levels. One may say that there is a bias towards citing the results of Russian scientists. For readers from Western countries, however, this is an advantage rather than a weakness, since it makes available material which is otherwise difficult to obtain.

In the reviewer's opinion the greatest value of the book is to be found in the first four of the book's six chapters: history; range of distribution and frequency of occurrence; morphology and dynamics; and luminosity. All these chapters are very comprehensive and very well presented—not surprising, since this is where the authors have their primary interest and competence. Chapter 5 on the nature of noctilucent clouds is not as complete and up to date as one might wish. The manuscript of the book was, however, finished in June 1969, and much of the information about mesospheric humidity has become available since then. The historical paper by Bates and Nicolet on water vapour in the high atmosphere clearly deserves due reference, and photochemical models with eddy transport, pointing at a humid mesopause, were also available.

This book will be a valuable reference for all those interested in noctilucent clouds.

Eigil Hesstvedt

Eigil Hesstvedt is Professor in the Institute of Geophysics, University of Oslo, Norway.

● In the review of *The Hydrolysis of Cations* (Wiley-Interscience; *Nature*, **263**, 802; 1976), the price was incorrectly quoted. This should read: \$34.95; £20.85.

● A UK edition of *The Poverty of Power* by Barry Commoner (for review, see *Nature*, **264**, 134; 1976) has been published by Jonathan Cape, London, at £5.50.

Drosophila genetics

The Genetics and Biology of Drosophila. Volumes 1a, b, c. Edited by M. Ashburner and E. Novitski. Pp. xv+486, xv+487-954, xii+955-1,427. (Academic: New York, San Francisco and London, 1976.) £16.80 per volume.

THE present three volumes represent a substantial part of an ambitious treatise dealing with the genetics, biology, development, evolution and ecology of *Drosophila* in the form of a series of articles contributed by specialists in the various subdivisions of this extensive field. They deal with the more strictly genetic aspects and display the great variety of analytical techniques which can be applied, especially in *D. melanogaster*. Ever since the early days of *Drosophila* genetics, presided over by the now legendary associates of T. H. Morgan, there has been a progressive development of ingenious ways of exploiting structurally altered chromosomes to investigate the properties of genes and chromosomes, often combined with supporting evidence from the ordered banding of the polytene chromosomes of the salivary gland. This methodology is dealt with in the articles according to their relevance to particular topics, but it is a pity there is no classified account of the principles of analysis and how they may be applied to different kinds of problem. This would have been valuable for newcomers to *Drosophila* and would have reduced the sense of discontinuity created by the rather arbitrary ordering of topics. Is it too much to hope that such an article could be included in a later volume of this series? Might the editors be persuaded to round off their labours with what could well be a *tour de force*?

In the total of some 1,400 pages the articles deal with such topics as chromosome aberration, conditionally expressed mutations, mosaic systems, gene conversion, meiotic mutants, distributive pairing, compound and ring chromosomes, altered segregations including segregation distortion, genetics of the Y chromosome, analysis of simple and complex loci, analysis of the proximal region of the X chromosome and of the fourth chromosome, position effect variation, mitotic recombination, the bobbed locus, radiation genetics, chemical mutagenesis, polytene chromosomes, together with reviews of *D. virilis* and *D. hydei*.

Merely to list these topics, which are generally covered in depth, is sufficient to indicate the wealth of information and conceptual diversity packed into these volumes. Naturally, almost all the theoretical framework has been strictly genetic in origin; ultimately it

will have to be reconciled with molecular evidence and interpretation, and a promising start has been made in that direction. Studies of the kinetics of extracted DNA, its appearance under the electronmicroscope, DNA-DNA and DNA-RNA hybridisation, the sequencing of satellites, *in situ* hybridisation to the polytene chromosomes and the characterisation of transfer RNA have already made a notable contribution. The elegant analysis of the bobbed locus described here is an excellent example of how the classic *Drosophila* methodology can be integrated with the analytical methods of nucleic acid biochemistry. Current developments in DNA sequencing will surely guarantee that the *Drosophila* genome will continue to be better known than that of any other eukaryote.

This interest in the use of the analytical tools provided by *Drosophila* is not confined to molecular biologists. In the past decade students of embryonic development and differentiation, cell lineage in organ formation, behaviour, and the biochemistry of gene action have discovered in *Drosophila* strategically significant situations worth investigating.

One of the pleasing features of this work is that the historical aspect is not neglected. There is an introductory chapter by C. P. Oliver, one of the *Drosophila* veterans, which outlines the

chronology of the major discoveries. In many of the articles there is comment on the origin of new interpretations or techniques and the reader is often thus reminded of the friendly exchange of stocks and ideas which has been, and remains, a traditional attitude among *Drosophila* workers.

It is obvious from the list of 44 contributors to the first three volumes of this series, that the centre of gravity of *Drosophila* research lies within the confines of North America, not overlooking some important European laboratories. It is a pity there are so few British laboratories in which the *Drosophila* lore flourishes. Perhaps these historic volumes will encourage some of our younger biologists to invade the field, provided they have a chance of reading them, none too certain in these days of dwindling library grants in view of the regrettably formidable price per volume.

It must have required immense effort, patience and tact on the part of the editors to marshal so many authors and ensure that the contributions were sufficiently similar in style and standard. They are to be congratulated on their altruistic devotion which will be gratefully acknowledged for many years to come.

Forbes W. Robertson

Forbes Robertson is Professor of Genetics at the University of Aberdeen, UK.

Marine pollution

Effects of Pollutants on Aquatic Organisms. (Society for Experimental Biology, Seminar Series 2). Edited by A. P. N. Lockwood. Pp. x+193. (Cambridge University: Cambridge, London and New York, September 1976). Hardcover £8.50; paperback £3.80.

THE overall trend towards physiological effects and turnover is apparent from most of the papers collected in this volume. Heavy metal storage, excretion and tolerance is covered comprehensively by Bryan with considerable experimental data on *Nereis*. The effects of cadmium on fish biochemistry is described by Larsson *et al.* Cautious comparisons are made with published work in the mammalian field.

Fish physiology is taken up further by Lloyd and Swift. Gas exchange and water balance are discussed with some emphasis on the care required in explaining observed abnormalities in fish kept in captivity. A quantitative approach towards histological studies in gills is presented by Hughes along with interesting information on fish respiration and circulatory mechanisms. Sup-

port is expressed for the tentative hypothesis of Lloyd, that increased ventilation rate by the gills increases the toxicity of poisons.

Organochlorine compounds are dealt with in a detailed review by Addison, in which the author makes several helpful suggestions for future work. The effects of hydrocarbons are covered in two papers. Corner *et al.* comprehensively cover zooplankton and fish. Vandermeulen and Ahern appropriately deal with the primary producers, being concerned with algal physiology. Both papers include experimental data on the effects of naphthalene on these organisms. Zooplankton and phytoplankton are discussed again by Reeve *et al.* who describe the first year's results obtained in the ambitious 'Controlled Environment Pollution Experiment'. Interesting comparisons with laboratory-acquired data are presented with particular reference to copper.

The wide coverage of this book, its readability and excellent index, will make it valuable for project hunters and established researchers alike.

G. C. Carney

Dr Carney is a Lecturer in the Department of Science at Bristol Polytechnic, UK.

Bare outlines of medicine

Molecular and Cellular Mechanisms in Disease. Vol. 1: Pp. xv+1-604+xxvi. Vol. 2: Pp. ix+605-1116+xxvi. By Julian L. Van Lancker. (Springer: Berlin and New York, 1976.) DM362, \$148.50.

AFTER some random reading here and there in the two volumes which make up this enormous monograph, I began to experience an extraordinary sensation of *déjà vu*. It was easy to trace this sensation to my memory of an earlier, equally enormous monograph on cellular pathology. The relationship between the two may be likened to that between the very 'with it' grandson of a highly respectable grandfather, with the grandson remaining very much a pillar of the family firm. Every generation of pathologists seems to produce one who cannot resist the temptation of trying to put down in one tome all knowledge that could possibly pertain to the subject, presumably in the hope of making it more comprehensible. This is in the tradition of Virchow and Cohnheim. In their day one man could still know enough facts in all the very different facets of pathology with sufficient accuracy to think about their interrelationship fruitfully. That has long since become impossible.

This book, therefore, demonstrates the case against a multi-topic textbook written by a single person. An almost unbelievable erudition is incapable of preventing errors of fact and distortions in interpretation, usually as a result of oversimplifications, which are serious enough to vitiate any supposed advantage arising from the unity of one man's outlook. In the arts a wide-ranging survey by one percipient mind may be singularly stimulating; thus, the *Outline of History* by H. G. Wells is for many an unforgettable intellectual experience. That this is still possible in the natural sciences is shown by such books as Jacques Monod's *Chance and Necessity* or Richard Dawkin's *The Selfish Gene*. But these are books of ideas, not compendia of information; and therein lies the difference between monographic success and failure.

In the preface the author says: "The book cannot be comprehensive because too little is known about the mechanisms of disease, and no-one could hope to master all available knowledge." So the puzzle is why the attempt was made to do just that. The comprehensiveness of the attempt is shown by the chapter headings: cellular sources of energy; determination of cellular specificity; inborn errors of metabolism; malnutrition; calcium and phos-

phorus metabolism; iron and bile pigment metabolism; blood coagulation; hormones; alterations of body fluids and electrolyte metabolism; cellular death and degeneration; blood lipids in arteriosclerosis and hyperlipidemia; radiation; inflammation; immunopathology; regeneration, hypertrophy, and wound healing; cancer.

The only way in which someone with much else to do can assess such a book is by reading carefully sections in which his own knowledge is as accurate as possible. Thus, the chapters on blood coagulation, hormones, blood lipids in arteriosclerosis and hyperlipidemia, and inflammation contain much useful, standard information, but any serious student would have to be provided with accurate, up-to-date reviews to correct impressions produced by the book. For example, many of the statements on page 410 are erroneous in fact or interpretation; on page 767 consideration of increased capillary permeability is entirely morphological and non-quantitative, and ignores all physiological and quantitative descriptions; on page 771 there are still discussions of "leukotaxine" and "exudine" which might have been assumed to have been buried long ago;

and on page 775 and beyond, the account of the prostaglandins is necessarily already out of date. Indeed, such a book is bound to be out of date by the time it appeared. The references seem to be mainly to the 1960s although some reach as close to the present as 1973 and 1974; and there are references to truly historical papers. There is no harm in that—on the contrary, students nowadays know much too little of the history of their subjects—as long as it does not result in underemphasis of modern developments.

According to the preface, the book is addressed to students of disease whether they are clinicians or researchers. "Hopefully, it will contribute to making medicine more of a science, guide the investigator in unexplored territory, and help the practitioner respond to the request of Macbeth, '... find her disease/And purge it to a sound pristing health'." It seems sad to have to conclude that this book will not achieve what the author hopes of it.

G. V. R. Born

Professor Born is Sheild Professor of Pharmacology at the University of Cambridge, UK.

Contemporary immunology

Manual of Clinical Immunology. Edited by N. R. Rose and H. Friedman. Pp. xxv+932. (American Society for Microbiology: Washington, August 1976.) Hardback \$20; paperback \$16.

THE burgeoning of immunology in the past twenty years has caused alarm to granting bodies and to its increasingly confused protagonists. The grass roots of this monster growth are to be found in methods of vaccination, blood group typing and the various serodiagnostic and classificatory methods of microbiology. Since the last war the successive discoveries of cell-mediated immunity, immunological tolerance, the recirculation of the lymphocyte, a function for the thymus and the fine structure of immunoglobulin molecules have effectively nourished the somewhat desiccated root disciplines and led to the rather top-heavy state of contemporary immunology.

The present volume is a blend of applications of the new immunology with up-to-date versions of some of the older approaches. It is sad that relatively little space is given to epidemiological studies, to vaccination technology and strategy, to the special problems of immunological

staging of cancer patients, to the quantitation of lymphocytes by reference to their origins and to parasitic diseases. It may be, however, that some of these topics are already well covered in other large books. The emphasis of the book is avowedly practical, and as a manual it is remarkably useful even if not comprehensive.

The book is split into eleven major sections covering fields such as "Transplantation immunology", "Immunoadassays", "Autoimmune diseases" and "Humoral immunity". There is a welcome section on the organisation and development of clinical immunological facilities. Each section consists of a number of papers by specialists in the field. It is claimed that enough information is given about techniques for an experienced technologist to carry out a procedure which was new to him (or her) without further search of the literature. As far as I can judge the chapters are clearly and concisely written with snags and deficiencies exposed to view.

It would be nice to see a book like this with the pages detachable on metal rings and with a visible index if it is really intended as a laboratory manual. The book is, however, good value.

A. J. S. Davies

A. J. S. Davies is Professor of Immunology in the University of London, working at the Chester Beatty Research Institute, London, UK.

announcements

Appointments

Professor C. C. Booth has been appointed by the Medical Research Council to succeed Sir Graham Bull as Director of the Clinical Research Centre, Northwick Park, Harrow.

Awards

The **Paul Martini Award** has been increased to 20,000 DM. The award is for development of scientific methods for evaluation in the clinical, pharmacological and therapeutic fields. Papers should be sent with six copies to the Medizinisch Pharmazeutisch Studiengesellschaft e.V., Humboldtstrasse 94, D 6000 Frankfurt. The competition deadline will be April 30, 1977.

Meetings

March 23–25, **Nuclear Physics**, Surrey (Institute of Physics, 47 Belgrave Square, London SW1).

March 23–25, **The Challenge of Change**, York (Institute of Information Scientists, S. Nichol, 'Rivendell', Southfield Road, Driffild, North Humberside).

March 26–April 5, **Genetics, Evolution and Ecology of Marine Organisms**, Venice (J. A. Beardmore, University College of Swansea; P. Battaglia, University of Padua).

March 28–April 1, **Chemistry in Our Lives**, London (The Chemical Society, Burlington House, London W1).

April 4–7, **Displays for Man-Machine Systems**, Lancaster (Institution of Electrical Engineers, Savoy Place, London WC2).

April 15–16, **Stadler Genetics Symposium**, Columbia, Missouri (117, Curtis Hall, University of Missouri, Columbia Mo 65201).

May 3, **Crop Protection**, Gent, Belgium (Ir. W. Dejonckheere, Agricultural Sciences, University of Gent, Belgium).

May 25–27, **The Testis**, Toronto (I. B. Fritz, University of Toronto, 112 College St, Toronto, Ontario M5G 1L6, Canada).

May 31–June 2, **Pharmaceutical Technology**, Paris (Association de Pharmacie Galenique Industrielle, Rue J. B. Clement 92290 Chatenay-Malebry, France).

June 1–3, **Frequency Control**, Atlantic City, New Jersey (J. R. Vig, US Army Electronics Command, Fort Monmouth, New Jersey 07703).

Person to Person

Surrey–Minneapolis, St Paul. 4-bedroomed house in Frensham near Farnham, Surrey, offered in exchange for similar sized house in Minneapolis, St Paul, from March 1977 for one year. Frensham is a village in delightful countryside within commuting distance of London by train. The Universities of Surrey and Reading are within 30-min drive. Contact Dr J. N. Gibbs, Dragon Lodge, Millbridge, Frensham, Surrey.

Wanted: copy of *The History of the Study of Landforms*, vol. 1, *Geomorphology Before Davis*, by R. J. Chorley, A. J. Dunn & R. P. Beckinsale (Methuen, London, 1964). Please state condition and price to Dr P. J. Smith, Department of Earth Sciences, Open University, Walton, Milton Keynes, UK.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

July 5, **Solar Energy Conversion**, London (P. T. Landsberg, The University, Southampton).

July 17–20, **International Metallographic Society**, Houston, Texas (IMSI, Evanston, Illinois 60204).

July 25–28, **Myxobacteria**, Norwich (J. H. Parish, Biochemistry, University of Leeds, UK).

August 15–17, **Endocrine Functions of the Testis**, Austin, Texas (Society for the Study of Reproduction, 113 North Neil St, Champaign, Illinois 61820).

August 21–September 3, **Genome Organisation and Function**, Spetsai, Summer School (H. G. Zachau, Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie, Goethestrasse 33, D-8000 München 2).

August 23–26, **Chemical Thermodynamics**, Ronneby, Sweden (IUPAC Conference, S. Sunner, Chemical Centre, Lund University, PO Box 740, S-22007, Lund, Sweden).

August 24–26, **High Polymers**, Ottawa (J. Roovers, 19th Canadian High Polymer Forum NRCC, Division of Chemistry, Ottawa, Ontario K1A 0R9, Canada).

September 1–2, **Cancer Immunotherapy and Prophylaxis**, Bucharest (Oncological Institute, P.O. Box 5916, Bucharest 12, Romania).

September 5–9, **Precise Electrical Measurement**, London (Institution of Electrical Engineers, Savoy Place, London WC2).

October 2–8, **Physical and Chemical Information Transfer in Regulation of Reproduction and Ageing**, Varna, Bulgaria (J. G. Vassileva-Popova, Biophysics, Bulgarian Academy of Science, 1113 Sophia, Bulgaria).

October 17–20, **European Society for Radiation Biology**, Liège, Belgium (R. Goutier, 32 Boulevard de la Constitution, B-4020 Liège, Belgium).

October 31–November 4, **Radioimmunoassay**, Berlin (E. H. Belcher, Medical Applications Section, IAEA, Kartner Ring 11, PO Box 590, A 1011, Vienna, Austria).

April 4–7, 1978, **Communications Equipment and Systems**, Birmingham (Institution of Electrical Engineers, Savoy Place, London WC2).

Reports and Publications

UK & Ireland–November

National Radiological Protection Board. NRPB R42: Radiological Protection Tests for Products which can Lead to Exposure of the Public to Ionising Radiation. By Marion D. Hill, A. D. Wrixon and B. T. Wilkins. Pp. 16. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1976.) [11]

Proceedings of the Royal Irish Academy. Vol. 76, Section B, No. 19: Bird Communities of some Killarney Woodlands. By L. A. Batten. Pp. 285–314. 87p. No. 20: Variation in Invertebrate Community Structure in Three Freshwater Habitats in Ireland, as Described by an Index of Species Diversity. By E. Fahy. Pp. 315–322. 42p. No. 21: A Woodland Population of Small Rodents (*Apodemus sylvaticus* (L.) and *Clethrionomys glareolus* Schreber) at Adare, Co. Limerick. By J. S. Fairley and J. M. Jones. Pp. 323–336. 63p. No. 22: Explosion Breccias and Related Features in the Leinster Caledonian Massif, South-East Ireland. By J. C. Brindley, L. N. Gupta and P. S. Kennan. Pp. 337–348. plate 12. 60p. (Dublin: Royal Irish Academy, 1976.) [21]

University College of Wales, Aberystwyth. Report of the Welsh Plant Breeding Station for 1975. Pp. 145. (Plas Gogerddan, Near Aberystwyth: Welsh Plant Breeding Station, 1976.) £1. [31]

Environmental Conservation in Action: Oil and Gas. (Shell Briefing Service.) Pp. 8. (London: Shell International Petroleum Co., Ltd., 1976.) [31]

A Correlation of Dinantian Rocks in the British Isles. By T. N. George, G. A. L. Johnson, M. Mitchell, J. R. Prentice, W. H. C. Ramsbottom, G. D. Sevastopulo and R. B. Wilson. Pp. 87. (Special Report No. 7). (London: Geological Society of London, 1976.) £3.50; \$7.50. [51]

Potato Marketing Board. Annual Report and Accounts 1976. Pp. 36. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1976.) [81]

The Family Planning Association. 44th Report and Accounts, 1975–1976. Pp. 29. (London: Family Planning Association, 27/35 Mortimer Street, W1, 1976.) [81]

The British Library. Science Reference Library. Periodicals on Ornithology held by the SRL. Pp. 14. (London: Science Reference Library, 1976.) [81]

Edwards Freeze-Drying Handbook. By Terence W. G. Rowe and John W. Snowman. Pp. 113. (Crawley: Edwards High Vacuum, 1976.) [81]

An Illustrated Key to Freshwater and Soil Amoebae with notes on Cultivation and Ecology. By F. C. Page. Pp. 155. (Scientific Publication No. 34.) (Ambleside, Cumbria: Freshwater Biological Association, 1976.) £2.50. [101]

EEC: Your Questions Answered—a Businessman's Guide, Pp. 129. (London: EEC Information Unit, Department of Industry, 1 Victoria Street, 1976.) [1111]

Jaguar V12 Engine: Its Design and Background, By Walter T. F. Hassan, Pp. 52. (Little Green, Richmond: Technical, Administrative and Supervisory Section, Amalgamated Union of Engineering Workers, 1976.) Members 30p; Non-Members 50p. [1511]

Land for Agriculture, (CAS Report 1.) Pp. 101. (Reading: Centre for Agricultural Strategy, The University, 1976.) £1.50. [1511]

Selected Annual Reviews of the Analytical Sciences, Vol. 4, Edited by L. S. Bark, Pp. v + 71. (London: The Chemical Society, 1976.) £9.50; \$19. [1511]

Queen Mary College, University of London, Annual Report, Session 1974/1975, Pp. 51. (London: Queen Mary College, 1976.) [1511]

Mining Research and Development Establishment, Annual Report, 1975/76, Pp. 20. (London: The National Coal Board, 1976.) [1511]

Whither R & D? (Proceedings of the 1976 Symposium held on 27th April at The Royal Society, London, Pp. 73. (London: Research and Development Society 47 Belgrave Square, SW1, 1976.) Members £3; Non-members £6. [1511]

The Nuffield Foundation, Thirtieth Report for the year 1975, Pp. 104. (London: The Nuffield Foundation, 1976.) [1611]

Third Report from the Select Committee on Science and Technology, University—Industry Relation, Session 1975-76, Pp. 96. (London: HMSO, 1976.) £1.15 net. [1711]

British Engine, Boiler and Electrical Insurance Co., Ltd. Technical Report 1976, Vol. XII, Pp. 80. (Manchester: British Engine, Boiler and Electrical Insurance Co., Ltd., 1976.) [1711]

The Leverhulme Trust, The Eighth Report of the Leverhulme Trustees, 1971-1975, Pp. 135. (London: The Leverhulme Trust, 1976.) [1711]

Ministry of Overseas Development, Annual Report of the Directorate of Overseas Surveys for the year ended 31st March 1975, Pp. 58 + 5 maps. (London: HMSO, 1976.) £2.40 net. [1811]

Wildfowl 27, Edited by G. V. T. Matthews and M. A. Ogilvie, Pp. 176 (Slimbridge: The Wildfowl Trust, 1976.) £2.75; \$8. [1911]

Research in The Open University, Pp. 63. (Milton Keynes: The Open University, 1976.) [1911]

British Overseas Trade Board, Report for 1976, Pp. 56. (London: British Overseas Trade Board, 1 Victoria Street, SW1, 1976.) gratis. [1911]

International Union of Pure and Applied Chemistry: Physical Chemistry Division, Commission on Colloid and Surface Chemistry, Manual of Symbols and Terminology for Physicochemical Quantities and Units—Appendix II: Definitions, Terminology and Symbols in Colloid and Surface Chemistry, Part II: Heterogeneous Catalysis, Adopted by the IUPAC Council at Madrid, Spain, on 9 September 1975. Prepared for Publication by Robert L. Burwell, Jr. Pp. 71-90. (Oxford and New York: Pergamon Press, 1976.) £2.75; \$5. [1911]

University Adult Education in London: A Century of Achievement, By John Burrows, Pp. x + 122. (London: University of London, 1976. Distributed by National Institute of Adult Education, De Montfort House, Leicester.) £1.25. [2211]

Proceedings of the Royal Irish Academy, Vol. 79, Section B, No. 23: On the Crystal Stability of Outgassed NiA Zeolites, By B. Coughlan, W. M. Carroll and W. A. McCann, Pp. 349-358, 57pp. No. 24: First Records of *Tricheta subviridis* Dutrochet and the Status of Other Arhynchodellid Leeches (Hirudinea) in Ireland, By C. S. Woods, Pp. 359-367, 45pp. No. 25: *Tanyastix stagnalis* (Linn.) in County Galway, New to Britain and Ireland, By R. Young, Pp. 369-378, 50p. (Dublin: Royal Irish Academy, 1976.) [2311]

Republic of Ireland, Annual Report of the National Drugs Advisory Board, 1975, Pp. 27. (Dublin: National Drugs Advisory Board, 57C Harcourt Street, 1976.) [2311]

Royal Observatory Bulletins, No. 181: Collected Observations of the Transits of Mercury, 1677-1973, By L. V. Morrison and C. G. Ward, Pp. 361-420. (Herstmonceux: Royal Greenwich Observatory, 1975.) £1.40 net. [2311]

Magnetism Letters, Vol. 1, No. 1, 1976, Edited by David E. Cox, Pp. 1-32, Subscription rates (per volume of six issues, post paid): Inside Great Britain £39, Elsewhere \$75.00/£43. (London, New York and Paris: Gordon and Breach, 1976.) [2311]

Commercial Assessment of Recently Introduced Potato Varieties: Report on Trials and Surveys 1976, Collated by R. M. Meredith, Pp. 21. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1976.) [2411]

Guy's Hospital: 250 Years, Edited by Clive E. Handler, Pp. vi + 232. (London: Guy's Hospital Gazette, 1976.) £2. [2411]

The Wash Water Storage Scheme Feasibility Study: a Report on the Ecological Studies, (Central Water Planning Unit), Pp. 36. (London: Natural Environment Research Council, 27/33 Charing Cross Road, WC2, 1976.) [2411]

The British Library, Report No. 5265 HC: Report of a Workshop on Multilingual Systems, Pp. 35. (Boston Spa, Wetherby: The British Library Lending Division, 1976.) £2.50. [2411]

Making the Best of It: Reconciling Ends, Means and Resources in Higher Education, (The Final Report of the Group for Research and Innovation in Higher Education), Pp. 38. (London: The Nuffield Foundation, 1976.) [2611]

Mineral Resources, Development and Technology, By Professor Henry E. Cohen (Inaugural Lecture 12 October, 1976, Royal School of Mines, Imperial College of Science and Technology), Pp. 13. (London: Royal School of Mines, Imperial College, 1976.) [2611]

Other Countries—November

Smithsonian Contributions to the Earth Sciences, No. 17: Occurrence, Distribution, and Age of Australite Tektites, By R. O. Chalmers, E. P. Henderson and Brian Mason, Pp. iii + 46. Smithsonian Contributions to Zoology, No. 233: Analysis of the Intergenic Relationships of the Australian Frog Family Myobatrachidae, By W. Ronald Heyer and David S. Liem, Pp. 29. (Washington, DC: Smithsonian Institution Press, 1976. For sale by US Government Printing Office.) [311]

United States Department of the Interior: Geological Survey, Bulletin 1385-E: Mineral Resources of the Eagle Cap Wilderness and Adjacent Areas, Oregon, By Paul L. Weis, et al., Pp. ix + 100 + plates 1 and 2. (Washington, DC: US Government Printing Office, 1976.) [411]

S. A. Sugar Association Experiment Station, Annual Report, 1975/76, Pp. 80. (Mount Edgecombe, Natal: South African Sugar Association, 1976.) [411]

United States Department of the Interior: Geological Survey, Bulletin 1415: Marine Gold Placers along the Gulf of Alaska Margin, By Erk Reimnitz and George Plafker, Pp. iii + 16. (Washington, DC: US Government Printing Office, 1976.) [811]

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